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Dera Ghazi Khan- 32200
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Hussain, F., S. S. Shaukat, M. Abid, F. Usman and M. Akbar. 2014. The effect of fungicides alone and in conjugation with chitin on the control of some fungal pathogens associated with chilli seeds. *World Appl. Sci*. J., **32(2):** 977- 985.

Book:

Hussain, F. 2015. *A manual collection, isolation techniques and identification of Fungi.* Vol: 1. Scholar's Press, Germany, pp 240.

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A contribution to the study of resource and community pattern in a Chir Pine Ecotonal Forest, Pakistan

Khadija Shafiq[a], Muhammad Hashim[a]*, Allah Bakhsh Gulshan[b], Eamon Bushra[a], Ishtiaq Ahmad[a], Adeela Altaf [c], Nazir Ahmad[a], Asia Bibi[a], Shaheen Qadir[a], Altaf Ahmad Dasti[a]

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Abstract

The forest type under consideration occupying Murree Hills and classified as tropical forests dominated with *Pinus roxburgii* (Chir Pine). These forests merge upward with temperate mixed conifer forests and downward with dry thorn forests. Study area is quite heterogeneous in topography and elevation ranges from 2200 m- 2600 m (a. s. l). It is located between 33°58.65 N´ to 73°22.60 E´. After orientation surveys of the area sampling sites were marked along an elevational transect. As a result of field work, the vascular flora reported in 35 stands contains a total of 72 species, belonging to 69 genera and 42 families. Most of soil cations and anions were significantly associated with vegetation variation along an altitudinal gradient. Current research work provides an integrated view of structure and composition of vegetation of area under consideration and also explains the link between resource pattern and community pattern across the altitudinal gradient.

Keywords: Ecological factors; Altitudinal gardient; Communiy pattern

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1. Introduction:

Variation in species diversity along environmental gradient is major topic of ecological investigation and has been explained by reference to climate, productivity, biotic interaction, and topographic heterogeneity (Gonzalez-Espinosa *et al.,* 2004; Qain and Ricklefs, 2004). Mountain ecosystems around the globe usually have diverse biological communities and high level of endemism, due to their topography and history (Gairola *et al.,* 2008). Hence the presence of different forest types is the indicator of diversity in climatic and edaphic factors. The plant communities of a region are function of time; however, altitude, slope, aspect, rainfall, and humidity play a significant role in distribution of plant communities and their composition (Ahmad *et al.,*2010; Rawat, *et al.,* 2010).

Himalayan alpines are possibly core of foundation of vital endemic taxa such as *Viola, Primula, Androsace* and *Saxifraga* (Altaf *et al.,* 2020; Sharma *et al.,* 2019). The community variation and constitution of alpine flora is controlled by difficult climatic and also edaphic parameters such as altitude, aspect, slope, latitude, rainfall and humidity play a role in the development of plant communities and their composition. Species richness shows a remarkable geographical deviation (Hashim and Dasti, 2019; Sharma *et al.,* 2019; Altaf *et al.,* 2020; BiBi *et al.,* 2020; Nazir *et al.,* 2020). Many climatic parameters beside elevational gradients are used to give explanation of deviation of species diversity (Peter *et al.,* 1997; Takyu *et al.,* 2002; Currie and Francis, 2004). Along elevational gradients all types of living organism's richness have been affected by atmospheric variables (Whittaker *et al.,* 2001) that manipulate the distribution of species along the elevation slope (Korner, 2000). These climatic factors directly and indirectly determined the range of individual species along. Discovering and understanding the trends in associations of biotic and abiotic component of an ecosystem is a critical field of ecological research work (Takyu *et al.,* 2002; Shaheen *et al.,* 2011; Saima *et al.,* 2018). The computer based statistical approaches and multivariate analytical programs help the ecologist to understand the effects of environmental factors on the whole group of species (Bergmeir, 2002). Such approaches have been used for vegetation studies of Pakistan (Dasti and Agnew, 1998; Wazir *et al.,* 2008; Saima *et al.,* 2009).

The changing environmental factors that form the layering or divisions of an ecosystem has been explained by the altitudinal zonation in the rocky region. Temperature, humidity, and soil composition are main variables that determine the altitudinal zones. The nutrient content of soils at different altitudes further complicates the delineation of altitudinal zones. Soils which have higher nutrient content maintain bigger trees and vegetation. The tropical rain forest areas, lower elevations show less terrestrial (Grytnes and Vetaas, 2002; Tuomisto *et al.,* 2003; Khan *et al.,* 2011).

Altitude is one of the most important parameters in shaping the diversity, because it strongly effects growing season associated with temperature, the accessibility of nutrients and soil moisture (Rawat *et al.,* 2010; Rahman et al., 2017). Most of the investigation is done on the spatial balance in the temperate and tropics regions (Aiba and Kitayama, 1997; Noor and Khatoon, 2013). Soil properties strongly influence plant growth (James *et al.*, 2003; Tuomisto *et al.,* 2003; Noor and Khatoon, 2013). Soil chemistry has affected plant species composition at different levels of salinity (Sharma et al., 1991) organic matter, pH and calcium (Abbadi and El-Ghani *et al.,* 2002). Elevation, aspects, and slope are three environmental factors affecting soil and climate (Hutchings *et al.,* 2000; Eshaghi *et al.,* 2009).

There is a modern trend in ecological research to investigate the relationship between biotic and abiotic components of an ecosystem (Khan *et al.,* 2011; Huo, *et al.,* 2014; Rhaman *et al.,* 2017; Sharma *et al.,* 2019). Vegetation is the expression of environment in a specific habitat at a specific time and hence needs to be properly studied in relation to its surroundings at both species and community level (Khan *et al.,* 2012b). Vegetation composition and structure are influenced by various natural and anthropogenic factors (Gairola *et al.,* 2008; Wazir *et al.,* 2008; Saima *et al.,* 2018).

The effects of environmental factors on species groups have been given by computer-based statistical and multivariate analytical programs that help the researchers to determine structure in the data set and then to analyses it (Bergmeier, 2002; BiBi *et al.,* 2020). Statistical programs decrease the complexity of data by classifying vegetation and then relating the results to environmental variables (Terbraak and Prentice, 1988; Khan *et al.,* 2011; Saima *et al.,* 2018; BiBi *et al.,* 2020). Classification also overcomes troubles of comprehension by summarizing field data in a low-dimensional gap with similar samples near together and dissimilar ones far away and these types of approaches are not often used in vegetation investigation of Pakistan (Wazir *et al.,* 2008; Malik and Hussain, 2008; Saima *et al.,*2009).

The information of the relationship between vegetation and environment in various forest types of Pakistan is based on descriptions of biological conditions from field experience. Comprehensive species composition and its response to the composite edaphic and topographic variations are poorly known because an incorporated view of structure and composition of temperate forests in Pakistan is still deficient.

1.1. Objectives:

- Distribution of Vascular plant species in a Chir Pine Ecotonal Forest, in Murree hills near Changla Gali, was analyzed to determine either plant species occurred in discrete communities or vegetation was continuous along the altitudinal gradient.
- To determine, how do the species abundance and diversity vary with altitude.
- To provide an integrated view of structure and composition of area under consideration.
- To explain the link between resource pattern and community pattern across the altitudinal gradient.

2. Materials and Methods:

2.1. Study Area:

The study site as located in Punjab Chir Pine (*Pinus roxburghii*) Forest in Murree hills near Changla Gali (33°58.65 north to 73°22.60 east.). The elevation of the study site ranged from 2100 - 2600 m (a.s.l.). *Pinus roxburghii* form the top canopy in these forests. In the center of its distribution there was usually little or no middle canopy except in depressions where the evergreen oaks and some of their deciduous associates may extend down from the temperate forest and trees of the tropical or semitropical deciduous forest spread up from below. The transition from the temperate forest was usually fairly sharp. Chir-pine forests represented an ecotone between dry subtropical broad-leaved forest (Potohar scrub vegetation) and the lower moist blue pine (*Pinus wallichiana*) forests. It provided a crossroad between tropical to moist temperate vegetation and thus harbor a variety of species of different origin.

2.2. Climate:

The climate of the area is not uniform, and this is mainly due to huge variation in altitudinal ranges. Summers are mild and winters extremely cold. Higher altitudes (about 1200 m a. s. l) receive snow in the winters from December to March, whereas June to middle September receives the heavy rainfalls of monsoon. The climate of the area ranges from wet temperate at the base of the mountains to permanently covered with ice and snow at the highest altitudes. The winters are long and very cold, and the summers are short and pleasant. The scarcity of metrological stations in the study area makes

impossible to give a detail account of the climate. The normal values for study area are given in Table 1. Rainfall data of the study area is given in Figure 1. The overall climate of the study area is subtropical climate with monsoon influenced and can be classified as Cwb type of climate according to Koppan rating.

Table 1. Mean, Average high, Average low and mean annual temperature in (°C) of Lower Chir Pine Forests, Changla Gali, Murree Hills.

Month	Mean Temperature (°C)	Mean Maximum Temperature (°C)	Mean Minimum Temperature (°C)
January	3.2	6.7	-0.3
February	7	7.5	0
March	8.3	12.2	4
April	13.5	17.9	9.1
May	17.4	22.2	12.6
June	21	25.8	16.3
July	19.4	23.2	15.7
August	18.6	22	15.3
September	17.2	21.4	13.1
October	14.4	19.1	9.7
November	9.8	14.4	5.2
December	5.7	9.8	1.6

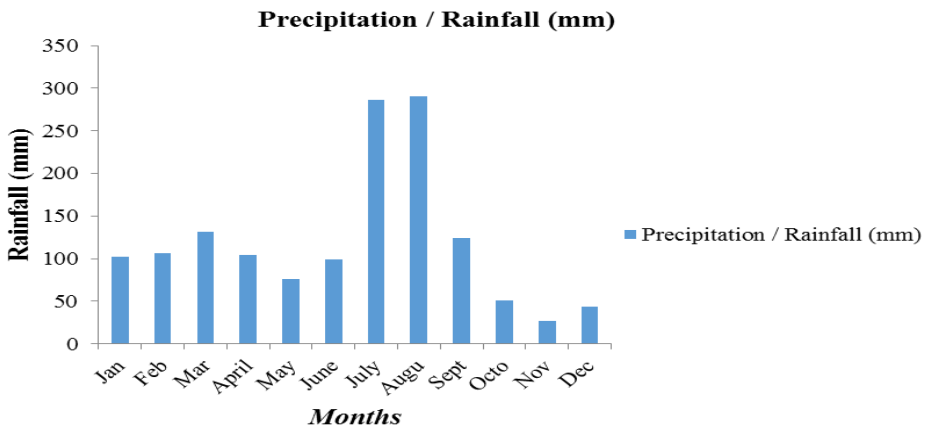

Figure 1. Average monthly precipitation (mm)of Lower Chir Pine Forests, Changla Gali, Murree Hills.

2.3. Vegetation sampling

After repeated survey, sites were selected along an accessible terrain running across the forest. A single transect was used instead of discrete plots as continuous sampling allows comparison of spatial changes between data sets, and assessment of whether turnover is spatially continuous or occur more rapidly at certain points (Tuomisto *et al.,* 2003a; Saima *et al.,*2009). Vegetation parameters were

recorded systematically in 30 Kilometer long transects. The direction of transect was from south to the north. At each Kilometer, 20 m^2 transect was established and vegetation parameters were monitored by placing 10 randomly placed 5m^2quadrats in each transect. All the vascular species were recoded except grasses because they form a little proportion of the total ground cover. All the stands were demarked within an estimated 2.0 m to the left side of transect during monsoon season (May to August 2017). Plants were identified in the field whenever possible, but in doubtful cases vouchers were collected. Specimens were collected carefully with their full structure (stem, leaves, flowers etc.). Samples of surface-soil were also collected at random point from all the sampling sites and were taken out for physical and chemical analysis following laboratory manuals of Richards, (1954) and Hussain (1989).

2.4. Vegetation analysis

The floristic data collected from 35 samples were simplified using multivariate analysis. For this purpose, a Multivariate Statistical Package was used (Kovach, 1999). Species richness and diversity index was estimated following Shannon diversity index (H) and the Evenness Index (J) (Magurran, 1988).

2.4.1. Vegetation Classification and Gradient Analysis

For classification of samples and species, cluster Analysis was applied on the presence/absence data. Samples were clustered, based on agglomerative clustering strategies (Hill, 1979; Causton, 1988) incorporating Euclidian Distance coefficient as a default option. In order to know the compositional pattern and to confirm the results of classification procedure of data simplification, ordination (Detrended Correspondence Analysis, DCA) was used. The DCA scores on axis 1 and axis 2 were used to determine the relationship between soil characteristics and distribution and assemblage of plant species by using Pearson's correlation. The calculation was made using MINITAB, a statistical computer package (Dasti & Malik, 1998; Saima *et al.,* 2018; BiBi *et al.,* 2020).

2.4.2. Soil Analysis

The pH of soil extract was recorded by using H.M 10 K digital pH meter (Richards, 1954). Electrical conductivity of the soil extract was obtained by using CM-30 ET digital electrical conductivity meter (Richards, 1954). Soil organic matter contents were obtained by Walkely and Black's method as described by Cramer, (2012). The total nitrogen in soil was determined by Kjeldahl method (Cramer, 2012; Saima *et al.,* 2018). Soil soluble cations and anions were determined b following (Richards, 1954).

2.5. Soil and Vegetation Correlation:

Differences in soil parameters between the different associations were estimated by suing the analysis of variance (ANOVA). The calculation was made with the help of MINITAB- a statistical computer package.

3. Results:

3.1. Vegetation Ecology

As a result of field work, the vascular flora of the study area contains a total of 72 species, belonging to 69 genera and 42 families. Angiosperms contributed a major share while pteridophytes and gymnosperms contributed little to the floristic richness of the area. The largest families were Lamiaceae and Asteraceae consisting of 8 and 4 species respectively. Diversity and species richness were estimated from the qualitative data obtained from each sampling unit (2 x 500 m), Observed values of Shannon diversity (H') in the study area ranged between 2.0 to 2.8, where high values indicate high diversity (Table 2). The values estimated for species richness (SR) ranged from 8.3 to 14.0, with high values representing high richness per unit area. The results (Table 2) demonstrated that species richness increased with decreased in elevation along the transect. The lower end of the transect exhibited maximum value (14.0), while minimum was recorded in the topmost end of the transect having species richness < 10 species per unit area. Similar elevation trends were observed for H and HB diversity indices which decreased with increasing elevation along the transect gradient.

Table 2. Species Richness and Diversity Indices: **SR=** Species Richness; **H=** Shannon Wiener Diversity index; **D=** Simpson Index; **B'=** Brillion Diversity index, obtained by Normal cluster analysis.

Association	SR	H'	D'	B'
A	16	2.72	0.94	1.87
B	15	2.63	0.93	1.79
C	14	2.54	0.92	1.75
D	13	2.52	0.90	1.72

3.2. Classification

Four main plant associations were recognized by the Normal Cluster Analysis. At first level of division the ten samples belonging to high altitude were separated from those located at low altitude. On second level of the division, samples belonging to the low altitude were separated from the rest by having *Calamintha vulgaris*, which was absent in all other associations located at high altitude. At high altitude associations were characterized by having *Anaphilus contorta, Euphorbia cornigera, Barbarea intermedia, Silene vulgaris, Delphinium unicatum, Napeta connate* which were absent at low altitude. These results suggested the importance of altitude in shaping the plant communities along the altitudinal gradient. At 4th level of cluster analysis seven associations were recognized of which the three (A–C) belongs to high altitude dominated by *Medicago falcate, Barbarea intermedia, Silene vulgaris, Delphinium uncinatum, Leucas molissima* and remaining four (D–G) were recorded at low altitude. The detail composition of all association is given in Table (3). Briefly description of associations presented below.

3.2.1. Association A: Pinus wallichiana - Aster falconeri community

This community type was found between 2100 and 2200 m (a. s. l). There were 51 species and 47 genera which were recorded in the study area. This community type was characterized by eight indicator species (Table 3). Out of 51 species, 74 % species were herbs. The field area was mostly covered by herbaceous plants. *Aquilegia pubiflora*, *Caltha palustris, Cannabis sativa, Lavatera*

cashmiriana, Skimmia laureola, Origanum valgare, Plectranthus rugosus and *Urtica dioca*, which were absent from all other associations recognized by Normal Cluster Analysis (Table 3). Among the herbaceous flora *Poa alpine, Caltha palustris, Prunella valgaris, Aster falconeri* and *Stachys sercea* were the dominant species. The soil of this association is characterized by having the high pH with a mean value of (7.0). Thus, showing slightly alkaline properties.

3.2.2. Association B: Quercus dilata - Oxalis corniculata community

This community was distributed at 2200-2300 m a.s.l. In this community 43 species belonging to 43 genera were recorded. This community was found on undisturbed places with depressions benefiting from runoff and is equally shared by six (13.95%) indicators species: *Calamintha vulgaris, Taxus wallichiana, Solanum nigrum*, *Polygonum aviculare*, *Myosotis alpestris*, *Lonicera quinque locularis,*which were altogether absent from rest (Table 3). *Arisaema jacquemontii, Oxalis corniculata, Sarcocca saligna, Impatiens sulcata, Impatiens edgeworthii, Trifolium ripens* were the dominant species in this community (Table 3).

3.2.3. Association C: Acer caesium - Capsella bursa-pastoris.

This community leads to altitude of 2300 m to 2400m. It contained total 40 species and 40 genera. Out of 40 species 21 were herbs (57.50%). This associations were prominent by having *Asplenium trihomonas*, *Lamium album, Acer caesium, Capsella bursa-pastoris*, *Anaphalis nepalensis* were absent from all other association. Among tree strata, 7 tree species contributed 17.5 % to the total floristic composition of this community. Among the herbaceous strata, *Impatiens sulcata, Bergenia ciliate, Trifolim ripens, Plantago major* were dominant species (Table 3). The common shrubs were, *Sarcococca saligna, Desmodium tiliaefolium, Berberis pachyacantha, Rosa macrophylla, Rubus biflorus, Strobilanthes wallichii, Vibernum cotinifolium, Clematis montana* and *Indigofera gerardiana*. The tree species of this community were (17.50 %) *Acer caesium, Ficus palmate, Pinus wallichiana, Populus ciliata, Aesculus indica* (Table 3).

3.2.4. Association D: Jasminium humile - Conyza japonica community.

This community was situated from the altitude of 2400m to 2550 m. It contains 47 species belonging to 46 genera which were identified. Out of 39 species 23 were herbs (58%). This community was characterized by three indicator species: *Ajuga parviflora, Conyza japonica, Achilla millefolium* which were altogether absent from the rest (Table 3). The dominant species named as *Brachiaria eruciformis, Impatiens sulcata. Bergenia ciliate Trifolium ripens*. The rare herb species of this association were *Arisaema jacquemontii, Myosotis scorpioides, Plectranthus, Aquilegia pubiflora, Sauromatum venosum, Achyranthes bidentate, Poaalpine, Aster tongolensis, Lamiumalbum, Prunellavalgaris, Dioscoria deltoids and Trifolim ripens.*

Table 3. Relative frequency of the species in each association from the normal cluster analysis, arranged in ascending order of DCA score, axis 1.

Species Name	Association A	Association B	Association C	Association D
Arisaema jacquemontii	6.50	4.46	-	0.74
Urtica dioca	5.69	-	-	1.48
Rumex nepalensis	4.07	1.27	2.63	2.96
Vibernum cotinifolium	4.07	3.18	0.88	3.70
Galium aparine	4.07	3.82	0.88	1.48
Geranium rotundifolium	4.07	2.55	2.63	1.48
Diospyros lotus	3.25	3.82	2.63	5.19
Rosa macrophylla	3.25	2.55	4.39	3.70
Pinus wallichiana	3.25	5.10	2.63	4.44
Hedera nepalensis	3.25	-	2.63	1.48
Imppatiens edgeworthaii	3.25	3.18	3.51	1.48
Myosotis scorpioides	3.25	0.64	-	0.74
Viola rupestris	3.25	1.27	3.51	4.44
Ficus palmata	3.25	3.18	5.26	2.22
Fragaria indica	2.44	1.91	0.88	2.22
Impatiens sulcata	2.44	3.18	5.26	2.96
Goodenia ovata	2.44	5.73	1.75	5.93
Origanum valgare	2.44	-	0.88	-
Oxalis corniculata	2.44	5.10	0.88	2.22
Epilobium angustifolium	2.44	1.27	-	-
Plectranthus sp.	2.44	-	0.88	0.74
Berberisppetiolaris	2.44	3.82	4.39	4.44
Plantago major	1.63	1.91	3.51	-
Polygonum aviculare	1.63	0.64	-	-
Aquilegia pubiflora	1.63	-	-	0.74
Sauromatum venosum	1.63	1.91	0.88	0.74
Scrophularialatefolia	1.63	1.27	-	-
Solanum nigrum	1.63	0.64	-	1.48
Dropteris remose	1.63	0.64	1.75	0.74
Stachys sercea	1.63	0.64	0.88	-
Lavatera kashmiriana	0.81	-	-	-
Achyranthes bidentata	0.81	1.27	-	0.74
Adiantum venustum	0.81	-	0.88	-
Aesculus indica	0.81	-	1.75	2.96
Quercus dilate	0.81	5.10	0.88	5.93
Micromeria biflora	0.81	1.27	1.75	1.48
Cannabis sativa	0.81	-	-	1.48

Catha edulis	0.81	0.64	-	-
Onichium sp.	0.81	-	0.88	2.22
Capsella bursa-pastoris	0.81	-	-	1.48
Paeonia emodi	0.81	1.91	-	-
Rubus biflorus	0.81	1.91	2.63	-
Poa alpine	0.81	0.64	1.75	0.74
Aster tongolensis	0.81	0.64	1.75	0.74
Strobilanthes attenuates	0.81	2.55	1.75	2.22
Brachiaria eruciformis	0.81	1.27	3.51	5.93
Indigofera gerardiana	-	0.64	0.88	1.48
Jasminium humile	-	1.91	6.14	-
Lamium album	-	-	-	0.74
Lonicera quinquelocularis	-	0.64	-	2.96
Populus ciliate	-	-	2.63	1.48
Prunella valgaris	-	0.64	2.63	0.74
Clematis montana	-	-	0.88	2.22
Nephalium sp.	-	-	4.39	-
Desmodium tiliaefolium	-	1.91	-	0.74
Dioscoria deltoids	-	2.55	-	0.74
Ajuaga bracteosa	-	-	2.63	-
Bergenia ciliata	-	-	4.39	-
Sarcococca saligna	-	4.46	-	1.48
Taxus wallichiana	-	0.64	-	-
Trifolim ripens	-	3.18	4.39	0.74
Skimmia laureola	-	-	-	0.74
Euphorbia wallichiana	-	1.91	-	-
Geranium wallichiana	-	0.64	-	2.22

3.3. Ordination:

The axis-1 and axis-2 accounted for 0.340 and 0.230 correspondingly of the total unevenness in composition of species, while 3rd and 4th axis have only 0.180 and 0.127, respectively (Table 4). This shows that first two axis when taken together can be considered as practically high-quality characterization of spatial distribution of species. The same seven groups were evident when the seven clusters produced by multivariate analysis which were plotted on the first two axis as scattered diagram (Fig. 2). The result depicted which in Figure 2 revealed the gradual change in community composition with partially overlapping fashion along the spatial gradient from high altitude to lower altitude. This pattern of site and species distribution suggested considerable differences in habitat along the altitudinal gradient. The plants groups delineated by the Cluster Analysis and confirmed by the ordination were not arbitrary but rather dictated by several edaphic factors. The relationship between environmental variables and the vegetation composition may be assessed by examining their correlations with DCA axis along the vegetation ordination.

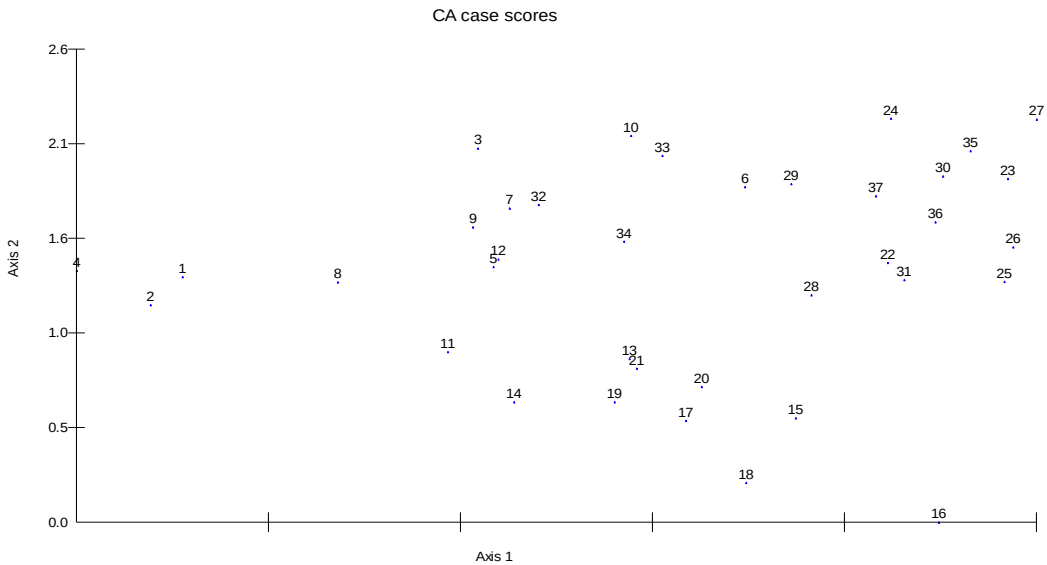

Figure 2. The Decorana (axes 1 and 2) plot of 21 samples from Sharan Valley. Stands are plotted individually, and zones are shown in which each of seven associations segregated to the cluster analysis as given in Fig 3.11 are found.

Table 4. Eigenvalues and Cumulative Percentage of DCA axes 1-4

Axes	Eigenvalues	Percent of Total	Cumulative Percentage
1	0.340	10.205	10.205
2	0.230	6.899	17.104
3	0.180	5.390	22.494
4	0.127	3.799	26.292

3.4 Soil Analysis

Almost all the soil variables included in the present investigation were significant ($p > 0.05$). Among the variable's altitude, showed the highest F value suggesting the importance of altitudinal difference in determining the composition of plant association along the altitudinal gradient. The number of species decrease with the decrease of altitude. At lowest altitude number of species were three-fold lower than at the highest altitude. The soils at the highest altitude were circum-neutral in reaction showed lowest value for EC. Other soil pH, O.M, N, K, P, Ca, Mg, CO_3, and HCO_3 showed no clear trends along the altitudinal gradient but exhibited significant difference among the plant's association. For example, soil of association C was poor in nutrients than the remaining associations identified by cluster analysis. The correlation between altitude diversity parameters were significant at $P< 0.05$ (Table 5). Examined the relationship between environmental variables and stand score along DCA axis 1 and 2 (Table 5). The results recommended the composition of the feeble flora is given by large number of soil parameters. However, their relative significance is complex to conclude. In the present investigation altitude has the overriding importance in determining the species composition. The

samples from the high altitude are located at the left-hand side, while the samples from low altitude are located on the other side of the ordination diagram. Besides this soil pH showed the strongest correlation with DCA axis 1 (Table 5). While the negative relationship between DCA axis 1 & pH suggested that pH decreases as one move from high altitude to low altitude (Table 5).

Table 5. Co-efficient of Pearson Correlation of factors between DCA first axis, DCA second axis, altitude, soil and environmental parameters

Parameters	Axis 1		Axis 2	
	Coefficient	Significance	Coefficient	Significance
Altitude(m)	-0.690	***	0.023	NS
No. of species	-0.041	NS	0.084	NS
pH	-0.701	****	-0.062	NS
E.C	-0.598	***	0.006	NS
O.M (%)	-0.726	***	-0.049	NS
N (ppm)	-0.521	**	0.076	NS
P (ppm)	-0.467	**	0.046	NS
K (ppm)	-0.780	***	-0.877	NS
Ca	-0.791	***	0.001	NS
Mg	-0.804	***	0.023	NS
Na	-0.525	***	-0.246	NS
Cl	-0.660	***	-0.024	NS

The physical and chemical soil data showed most of the edaphic variables in present investigation showed significant differences among these groups (Table 6). Soil pH, EC, organic matter, phosphorus, potassium and calcium etc. appeared to be the most important parameters in determining the differences in the substrata of plants groups and showed high F-Values (5).

Table 6. Analysis of variance (Anova) of different parameters

Source	DF	SS	MS	F	P
Altitude(m)	3	417538	139179	136.33	0.000
No. of Species	3	21.4	7.1	0.54	0.655
pH	3	1.725	0.575	2.80	0.055
E.C (mS/cm)	3	1.320	0.440	1.89	0.150
O.M	3	2.602	0.867	4.80	0.007
N	3	0.304	0.101	2.10	0.119
P	3	156.6	52.2	3.75	0.020
K	3	0.124	0.041	6.01	0.002
Ca	3	0.056	0.018	6.39	0.002
Mg	3	0.012	0.004	5.61	0.003
Na	3	11.62	3.875	4.49	0.009
Cl	3	0.308	0.102	3.76	0.020

The electrical conductivity values were high (2.52) as compared to other associations. Organic matter, Nitrogen, Phosphorous, Potassium, Calcium, Magnesium, and Chloride were high as compared to other associations. It contains high value of Sodium than any other association delineated by Normal cluster analysis. All other nutrients showed intermediate values. The soil of association exhibited a mean value of pH about 6.61 which showed slightly acidic properties. The association was

characterized by having high organic content of (1.254). The soil is characterized by having electrical conductivity of (2.17 ms/cm), Mg (0.237), and a pH of (6.50) as shown in the Table (Table 7). The soil of this association was quite poor by having high organic content (2.083), pH (6.41), phosphorus (12.44), Potassium, Calcium and Chloride were low as compared to other associations (Table 7). The soils of low altitude are characterized by having low concentration of Potassium and high concentration of sodium chloride (Table 7).

Table 7. Mean and Standard deviation of parameters of all associations

Source	Statistic factor	A	B	C	D
Altitude	Mean	2250.0	2350.00	2435.00	2528.9
	St.Dev	33.2	27.39	24.49	39.50
No. of Spp.	Mean	14	15	15	14
	St.Dev	3.683	4.738	2.872	2.712
pH	Mean	6.9559	6.6138	6.5089	6.4122
	St.Dev	0.5157	0.4470	0.3837	0.4382
E.C	Mean	2.5182	2.0856	2.1738	2.0833
	St.Dev	0.4311	0.4685	0.5728	0.4702
O.M	Mean	1.6936	1.2613	1.2544	0.9867
	St.Dev	0.5173	0.2120	0.5068	0.3394
N	Mean	0.3371	0.1412	0.137	0.137
	St.Dev	0.3992	0.0119	0.0014	0.004
P	Mean	17.455	13.000	15.125	12.44
	St.Dev	3.532	2.345	6.128	1.870
K	Mean	1.7782	1.6589	1.6663	1.630
	St.Dev	0.1216	0.0779	0.0256	0.057
Ca	Mean	0.554	0.477	0.471	0.461
	St.Dev	0.0749	0.059	0.03790	0.01356
Mg	Mean	0.2790	0.2444	0.2377	0.2362
	St.Dev	0.03562	0.03167	0.00916	0.01922
Na	Mean	11.475	11.500	10.422	10.311
	St.Dev	1.089	0.926	0.897	0.722
Cl	Mean	0.751	0.587	0.545	0.544
	St.Dev	0.2096	0.1950	0.0278	0.1390

4. Discussion:

Most of the edaphic factors included in the present investigation were associated with distribution of species in the study area. In terms of correlation, rank of factors was altitude > Mg>Ca> Percent organic matter> pH> chloride> EC> Na and Phosphorus. All these factors are significantly correlated with DCA axis 1. Two factors seem to be operative i.e., altitude and substrate that determine the boundaries and composition of plant communities across the landscape. Apart from the fact that edaphic variables are indeed relevant for explaining the main vegetation types, the correspondence between the results of cluster analysis and DCA planes permitted direct interpretation of course of stand data in DCA plane in relation to soil variables. The four groups produced by cluster analysis are plotted on the first two axes as scattered diagram. The ordination axes may represent in some way the major substrate influence which affect the stand in these data and have been used to discuss the overriding features of the environment. The importance of altitude as an environmental factor effecting plant assemblage is not surprising, considering its close correlation with rain fall and re-distribution of

rain fall water (Saima *et al.,* 2009; Dasti *et al.,* 2010; Saima *et al.,* 2018). The analysis and assessments of pattern and zonation along the first ordination axis suggest that the most important environmental gradient and boundaries across landscape are associated with down slope movement of water and soil chemistry. If this assumption is true, then it is suggested that some combined effect is important in determining the distribution pattern of plant species. This assumption is supported by the fact that all species show spatial distribution with relation to soil factors over relatively short environmental gradient in wet temperate coniferous forest. The changes in floristic composition along the altitudinal gradient have been reported by several workers (Grubb, 1977; Lieberman, 1987; Proctor *et al.,* 1988; Takyu *et al.,* 2002). Topography affects the edaphic conditions that govern diversity and species richness, spatial variations in the abundance of plant species. Several studies suggest that diverse site conditions contribute to the maintenance of species richness (Clark *et al.,*1997; Tuomisto *et al.,* 2003; Kubota, 2004). The complex gradients in edaphic conditions that are associated with topography (Provide an opportunity to assemble diversity of species) are important factors that determine the structural/compositional variations in plant communities.

Among edaphic factors change in soil nitrogen content along the altitude have overriding importance in determining the pattern of species distribution over the landscape. This agrees with the findings of Vitousek *et al.,* (1997); Bobbink *et al.,* (1998). Soil nitrogen was significantly higher in typical elevated terrain This correlation with altitude confirms the findings of Dasti & Malik, 1998 who reported higher nitrogen content in such terrain i.e., that is in upper mountain soil then the soil of lower altitude. The results suggested that altitude play significant role in determining the variations in soil properties in terms of soil anions and cations, organic matter, soil pH and overall soil electrical conductivity. The concentration of cations, anions and other soil parameters and organic matter often showed a steady decline with the increase in elevation from association A located at lower elevation than the association D located at higher elevation. Consequently, soil cations such as P, Ca^{++}, K^{+}, and Mg^{++} were consistently greater in association A than association D. Although the concentration of cations content showed constantly negative correlation with DCA axis 1 suggested a continuous decrease in cations with increase in altitude.

5. Conclusion:

Consequently, the plant assemblage in wet temperate forest determines by the soil chemistry. The reason for this correlation must be hydrological. Particularly the pattern of run –off generation and redistribution of soil nutrients of the study area and probably are common in many mountainous terrain (Wazir *et al.,* 2008). Thus, at lower altitudes high diversity and species richness might be due to higher concentration of cations. This might be true for the mountain vegetation and most typical of mountain in wet temperate forests. These results are consistent with other studies on diversity-altitude relationship (Dasti & Malik *et al.,* 1998; Wazir *et al.,* 2008). The relative magnitude of correlation demonstrate that elevation play a dominant role in determining the distribution of species. Support for this interpretation comes from both the ordination and classification. These groups delineated by the cluster analysis are correlated most closely with altitude and topography.

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Medicinal importance of few plants from moist temperate forest of West Himalaya, Pakistan

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Abstract

Present study was conducted in Ayubia National Park, which is in District Abbottabad, Khyber Pakhtunkhwa, Pakistan. The study area is a part of Himalayans moist temperate forest with a high diversity of vulnerable plant and animal species. A total of 65 species, belonging to 62 genera and 39 families were recorded. Angiosperms contributed a major share while Pteridophytes contributed little to the floristic richness of the area. The study revealed that 45 plants species belong to 34 families are used in folk medicinal system. The medicinal plants are mostly used to cure amenorrhea, skin allergies, and leucorrhoea, as abortifacient, post-delivery pain, dandruff, eczema, tonic after delivery and for breast milk secretion. All these herbal medicines belong to 65 herbaceous ground floras, 7% shrubs, 21% trees and 7% climbers. The present investigation was intended to describe the knowledge and significance of medicinal flora and their traditional uses in daily life.

Keywords: Medicinal plants; Ayubia National Park; Moist temperate forest; Western Himalaya

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1. Introduction:

Ayubia National Park (Ayubia) is a protected area of 3,312 hectares situated in Abbottabad District, Khyber Pakhtunkhwa province, northern Pakistan. It was declared a national park in 1984. Ayubia was named after the late Muhammad Ayub Khan (1958 – 1969), second President of Pakistan. Ayubia National Park is located between 33°51′54.83″N to 73°8′19.57″E. The area supports temperate coniferous forest and temperate broadleaf and mixed forest eco-region habitats, with an average elevation of 2,400 m (a. s. l). The topography of the area is characterized by series of arduous mountains with very steep slopes. The bed rocks are sedimentary comprising of limestone, shale and sandstone ranging in age from Triassic to Eocene. The soil is mostly loamy with a good mix of sand and gravel (Saima *et al.,* 2009).

Climatically the area was moist temperate continental type with cold winters and mild to pleasant summer (Saima *et al.,* 2010; BiBi *et al.,* 2020). The minimum temperature in winter season falls well below the freezing point. The mean annual rainfall is above 1,500mm. The precipitation received in the form of snowy winters. Snowfall during winter may accumulate to 3–7m at various places. Most of the rainfall is received during monsoon period from July to August while May, June, September, and October are the driest. The southwest aspect facing the monsoon winds has a much higher and better distributed rain fall than the opposite northeast aspect, which is rain shadow, Relative humidity measured range 60–70% (Khan, 1998).

The average annual temperature in Murree is14°C. At an average temperature of 21.0°C, June is the hottest month of the year. The lowest average temperature in the year occurs in January, when it is around 4°C. The average mean minimum and maximum temperature is 10°C and 18°C, respectively. November, December, January, and February remain the coldest months while the other months are moderate with pleasant temperature. The study areas are located broadly in wet temperate forest climatic zone, with long, frozen winters and short, cold summers; in early autumn, chilly winds bring temperatures down. Precipitation is received year-round with two maxima, first one during winter and second one at summer July-August. Monthly report of precipitation is given in Figure 3. Total mean annual precipitation is 1,29mm. The average depth of snow accumulation in winter is about 1.5 m and may be more on mountain peaks. Estimated mean precipitation ranges from 900 mm to 1350 mm (Khan, 1989; Hashim and Dasti, 2019).

Pakistan being blessed with rich plant biodiversity represents more than 6,000 flowering plant species (Shinwari, 1996). The plant species confined to hilly areas (Northern side) of Pakistan contributed main part of biodiversity and provided a strong association between plants and man (G.M. Shah *et al*., 2013). Medicinal plants used by the local people ethno botanically are of great importance that is the reason a lot of people are engaged in the trade of important medicinal herbs, shrubs, and tree species in and outside the country. The field of Ethno-botany in Pakistan is now not that virgin as it was in early 90's. A lot of papers have been published and more work has to be done in the future (Shinwari *et al.,* 2000). According to W.H.O., in Pakistan more than that 60% of the population used herbal medicines prescribed by traditional practitioners (Hamayun *et al.*, 2003-2006).

1.1. Objectives:

- The present investigation was intended to describe the knowledge and significance of medicinal flora and their traditional uses in daily life.
- To explore the medicinal flora in the core of moist temperate forest of West Himalaya, Pakistan.
- To promote the awareness of medicinal flora and its traditional uses in local community.
- To save and conservation of the medicinal flora from over grazing.

2. Materials and Methods:

2.1. Study of site area:

The study area was selected Ayubia National Park (Ayubia) situated at Abbottabad. The area has bulk quantity of medicinal flora and temperate coniferous forest with broadleaf as well as mixed forest eco-region habitats, with an average elevation of 2,400 m above the sea level.

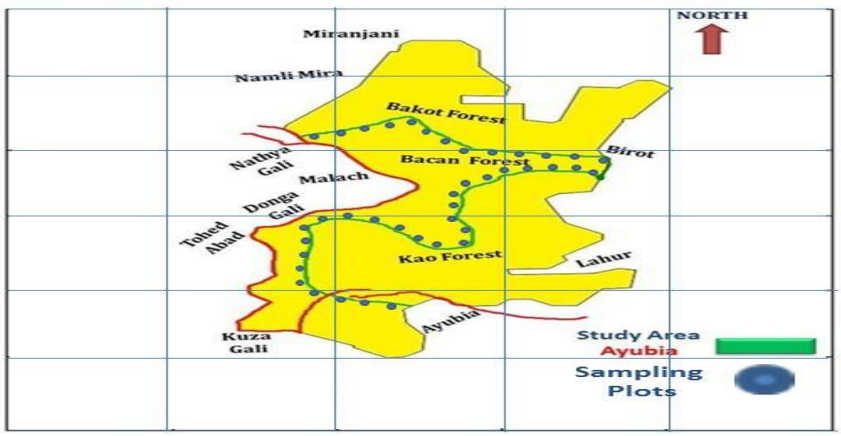

Figure 1: Map of the Study area Ayubia National Park

2.2. Sampling Method:

The brief study was conducted during the month of June to September. The plant specimens were collected, pressed, dried and identify with the help of available literature of Nasir and Ali, (1970) and Saima *et al.* (2010). The voucher specimens were deposited in Herbarium, Bahauddin Zakariya University Multan, Pakistan. The Questionnaires were made for the interviews of the people which included the information about the informants, the plant used by them, for what illness the plant is used. A total of 45 plants belonging to 34 families were studied for ethno-medicinal uses.

3. Results:

The results are presented on basis of ethno-medicinal uses. Each plant species is provided with its, Botanical name, and author citation followed by family (which it belongs), Vernacular name and Life form (Habit), lastly in brief the use of plant or plant part with its mode of preparation. The plants species sequenced alphabetically (Table 1). Plants such as *Artemisia roxburghiana, Ajuga bracteosa, Agrimonia eupatoria, Aquilegia pubiflora, Androsace rotundiflolia, Bergenia ciliata, Cedrus deodara, Cuscuta reflexa, Ficus palmate, Galium aparine, Impatiens bicolor, Oxalis corniculata, Podophyllum emodi, Potentilla nepalens, Polygonum amplexicaule, Quercus dilatata, Silybum marianum, Skimmia laureola, Solanum nigrum, Taxus wallichiana, Taxus wallichiana, Urtica dioica, Verbascum Thapsus, Viola canescens* and *Vitex negundo,* are multipurpose medicinal plants being significantly used for curing

purpose of diverse diseases. It is expected that this information will be useful for pharmaceutical industries and research students.

Table 1. Medicinal Plants Used by people of Ayubia National Park, Abbottabad District, Khyber Pakhtunkhwa, Pakistan.

S. No.	Scientific Name	Family	Habit	Plant Part Use	Ethno-medicinal Use
1	*Abies pindrow* Royle	Pinaceae	Tree	Rhizome & Leaves	Ulceration, Antiseptic & anticaceous
2	*Achillea millefolium* L	Asteraceae	Herb	Whole Plant	Skin Diseases, fever & Cold
3	*Ajuga bracteosa* Wall. ex Bth	Lamiaceae	Herb	Whole Plant	Amenorrhea
4	*Agrimonia eupatoria* L	Rosaceae	Herb	Leaves	Fodder, Fever & Gastric
5	*Aquilegia pubiflora* Wall. ex Royle.	Ranunculaceae	Herb	Seeds	Female Sexual Disorders
6	*Artemisia roxburghiana* Wall.ex Besser	Asteraceae	Herb	Leaves	Respiratory Diseases & Anti inflammatory
7	*Adiantum venustum* D. Don	Pteridaceae	Herb	Whole Plant	Tonic & Intestine
8	*Androsace rotundiflolia Hardw*	Primulareaceae	Herb	Leaves	Irregular Menstruation
9	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Herb	Leaves & Roots	Anticancer, anti-inflammatory, respiratory disorders, sexual diseases, and eyes problems
10	*Canabis sativa* Lamk.	Cannabinaceae	Herb	Leaves	Stimulant
11	*Cedrus deodara* (Roxb. ex Lamb.) G. Don	Pinaceae	Tree	Wood	Skin Diseases, Lactation, Ulceration, Pulmonary disorders, Urinary Diseases & Improve complexion
12	*Cuscuta reflexa* Roxb.	Cuscutaceae	Climber	Whole Plant	Eczema & Scabies
13	*Dryopteris ramosa* (Hope) C. Chir	Dryopteridaceae	Herb	Leaves	Tonic and Gastric Problems
14	*Dipsacus inermis Wall.*	Dipsacaceae	Herb	Leaves	Fodder, eye diseases
15	*Diospyros lotus* L.	Ebenaceae	Tree	Fruit	Amulet
16	*Ficus palmata* Forssk.	Moraceae	Tree	Leaves and stem bark	Vomiting, astringent, swelling, motions, asthma
17	*Euphorbia wallichii* Hook f.	Euphorbiaceae	Herb	Stem	Gastric problems & Skin Diseases
18	*Galium apparine* L.	Rubiaceae	Herb	Whole Plant	Jaundice, Antiseptic & anticanceous
19	*Geranium wallichianum* D. Don ex Sweet	Geraniaceae	Herb	Root	Tonic After Delivery, Strengthening muscles & bones

20	*Hedera nepalensis* K. Koch	Araliaceae	Climber	Leaves	Diabetes
21	*Impatiens bicolor* Royle	Balsaminaceae	Herb	Fruit & Leaves	Diuretic & Cooling effect
22	*Impatiens brachycentra* Kar. & Kir.	Balsaminaceae	Herb	Leaves	Fodder
23	*Juglans regia* L.	Juglandaceae	Tree	Bark	Toothbrush
24	*Jasminum humile* L	Oleraceae	Shrub	Roots & Flowers	Tonic & Intestinal Diseases
25	*Oxalis corniculata* L	Oxalidiaceae	Herb	Leaves	Improve mouth taste (Pregnant Woman)
26	*Polygonum amplexicaule* D. Don.	Podophylaceae	Herb	Leaves	Fever & diarrhea
27	*Paeonia emodi Wall.* Ex Hk.	Paeoniaceae	Herb	Root, Fruit & seed	Fever, Gastric Disease and Headache
28	*Podophyllum emodi* Wall. ex Royle	Podophylaceae	Herb	Fruit	Liver Disorders & Tonic
29	*Potentilla nepalensis* Hk.	Rosaceae	Herb	Root	Gastric problems, anti-inflammatory & Skin Diseases
30	*Populus ciliata* Wall.	Salicaceae	Tree	Leaves & Stem	Fodder
31	*Podophyllum multiflorum* (L.) All.	Polygonaceae	Herb	Rhizome	Copped Skin
32	*Quercus dilatata* Lindl.	Fagaceae	Tree	Leaves, Stem & Seed	Gastric, Urinary diseases
33	*Quercus incana* Roxb.	Fagaceae	Tree	Leaves, Stem & Seed	Gastric, Urinary diseases
34	*Rubia cordifolia* DC.	Rubiaceae	Climber	Whole Plant	Vagina Inflammation
35	*Rumex nepalense Spreng.*	Polygonaceae	Herb	Leaves	Constipation (During Pregnancy)
36	*Silybum marianum* (L.) Gaertn.	Asteraceae	Herb	Leaves & Root	Gastric, Anti- inflammatory and fever
37	*Solanum nigrum* L	Solanaceae	Herb	Leaves	Gastric, Skin & Respiratory
38	*Skimmia laureola* (DC.) Sieb. Zucc. Ex Walp	Rutaceae	Shrub	Leaves	Cough, Cold, Fever & Insecticide
39	*Tagetes erecta* L	Asteraceae	Herb	Leaves	Irregular Menstruation
40	*Taxus wallichiana* Zucc.	Taxaceae	Tree	Leaves & Wood	Induce menstruation, Anticancer, Pneumonia and Abortification
41	*Urtica dioica* L	Urticaceae	Herb	Whole Plant	enhance Lactation, anemia & Menorrhagia
42	*Verbascum thapsus* L	Scrophulariaceae	Herb	Whole Plant	Emollient, Cardiac & Fever
43	*Viola canescens* Wall.	Violaceae	Herb	Whole Plant	Respiratory Track, Gastric, Fever & Anti-inflammatory
44	*Vitex negundo* (L.) Moldenke.	Verbenaceae	Shrub	Whole Plant	Skin, Fever and Respiratory diseases

45	*Wulfenia amherstiana* Benth.	Scrophulariaceae	Herb	Whole Plant	Fodder

4. Discussion:

The present ethno-medicinal research provide information on medicinal uses of the 45 plants species belongs to 34 families. Most of species are quite effective remedies for different diseases like fever, gastric problems, ulcer, diabetes, female menstrual disorders, and respiratory disorders (Aneel *et al.,* 2001; Raja *et al.,* 2020). The area is under pressure because of illegal settlements, fires, and extensive tree cuttings, urban encroachment, and pollution (Qureshi *et al.,* 2009; Raja *et al.,* 2020). The conservation of biological diversity is one of the national and international needs to save our natural wealth. A decline in the total area of natural vegetation as a source of supply for medicinal plants has occurred partly as a result of removal of natural forests for agricultural land and partly due to commercial overexploitation of the medicinal plants themselves. Medicinal plants are collected for domestic use and also sold by poor people to earn money. Conservation of medicinal is very important, as most of these plants have been reduced to large extent and even some has disappeared from the area. With the growing interest in medicinal plants, both in the country and abroad, it is necessary to develop a long-term strategy to conserve and sustainably harvest these plant products (Sher and Hussain, 2009; Abbasi *et al.,* 2010). Therefore, immediate measures should be taken for in situ and ex situ conservation of these species in the district. The task should be undertaken not only by government but also by the non-governmental organizations (NGOs). More forest areas should be protected by declaring them as sanctuaries and reserve forests.

5. Conclusion:

The current research work described that much of medicinal plant species are used by indigenous people of the area under consideration to treat various infirmities in term of primary health issues. The indigenous community significantly dependent on traditional medicine, However the modern health-care services are available, which designates the imperatives of plant based traditional methods. Our research outcomes provide baseline data to establish a connection between the traditional health practioners and scientific communities, which could be more important in novel drug discovery. Furthermore, ethnobotanical data is of significant value for conservation managers and policy makers for sustainable management of medicinal plant species, which are under threat due to over exploitation. In Ayubia National Park, the vegetation is extensively being impoverished by the people mainly in the form of fuel wood, fodder, ethnomedicinal and grazing of animals. Reserves have been crucial for preserving species and habitats. Therefore, the area of Ayubia National Park was declared as protected by the Law in which human activities are restricted or prohibited. Protected areas are generally rich in the natural flora.

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Species Richness and Community Composition along Glaciated Alpine Lake, Pakistan

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Abstract

The study area is comprised of catchment area of glaciated lake locally called lake Saif-ul-Malook. The vegetation was studied along the altitudinal gradient located between 2500 m a.s.l to 3500 m a.s.l. and represent the mountain ranges of Western Himalaya. Overall vegetation may be classified as grass-scrub distributed along the rim of a glaciated lake (Saif-ul-Malook). From the study area a total of 91 plant species belonging to 75 genera and 37 families were recorded. Angiosperms contributed a major share in the study area. Among the Angiosperms, 36 families comprised of 90 species were recorded. Among these species dicotyledonous species had the largest share (94.5%) followed by monocotyledonous species which contributed very minor share (4.4%) to the floristic riches of the area. Data were analyzed by multivariate statistics including Cluster Analysis, Detrended Correspondence Analysis (DCA) and spearman correlation coefficient to detect the relations between altitudinal and environmental factors with the composition and structure of plant communities. DCA axes 1 and 2 were used for data interpretation. The three groups produced by cluster analysis were plotted on the first two axes as scattered diagram. There were significant differences in the floral composition as well as the edaphic factors along the altitudinal gradient. The present research work showed an integrated effort to analyze and discuss ecological community attributes, as well as their correlation with environmental variables, using multivariate analyses.

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Keywords: Ecological factors; Altitudinal gardient; Communiy pattern

1. Introduction:

The Naran valley which is occupying on the extreme boundary of the Western Himalayan range and expresses a prominent vegetation types relating the floras of Irano-Turanian and Sino-Japanese regions of the Central Asia (Ali and Qaiser, 1986; Khan *et al.,* 2011). Geologically, the Naran valley present on the extreme edge of Indian plate where it collides against the Kohistan is of Asian (Eurasian) plate (Wazir *et al.,* 2008). Phytogeography of Himalayan Mountains has been studied by a number of forest ecologists and most of information is available mainly on Eastern and Central Himalaya (Kharkwal *et al.,* 2005). While biogeographic, floristic, qualitative, and quantitative information about areas is incomplete. The flora and fauna of this valley comprises hundred species of animals and plants in which most species represent the vast diversity of Himalayan range and numbers of endemic species were also include. In this study area the floristic richness is noticeable as compared to remaining part of the Himalayan Range. This is considered as one of chief phytogeographical territory of the country. The ecological feature of this valley includes position of particular geography, promoted plant diversity and presence of meadows in alpine and sub alpine range (Altaf *et al.,* 2020).

The mountainous tracts have very difficult topography and inaccessibility. So, studies of vegetation present on it are very difficult. That is why the survey of flora and vegetation structure was incomplete. Classification of natural ecosystem into its communities and habitat types of natural recourses is important for long term management. Ecologists are always trying to know the variation in species richness and diversity along the ecological gradient (Vetaas and Grynes, 2002). Understanding and recognition of the relationship among abiotic and biotic component of mountain ecosystem is a critical and diverse branch of ecological research (Tavili and Jafari, 2009; Bibi *et al.,* 2020).

Classification of plants communities also reduces the problems of interpreting and summarizing the data obtained from field survey (Khan *et al.,* 2013). Previous research information about the vegetation structure and community composition is fragmentary and depend on qualitative description of the vegetation (Kharkwal, *et al.,* 2005). In mountainous forest ecosystem, elevation plays important role in determining the plant communities (Saima *et al.,* 2018). At high altitude, strong winds and snow pressure have great influence on species structure and composition (Vazquez and Givnish, 1998; Saima *et al.,* 2018; Hashim and Dasti, 2019). Alpine's regions show variations widely.

The climatic heterogeneity is considerable each year, especially in precipitation and snowfall, whereas soil contrasts not in depth, but also in reactions, soil cations and anions exchange capacity, soil edaphic properties (Shaheen *et al.,* 2016). The soil heterogeneity plays a further important basis for shaping vegetation pattern in this climate condition than climatic variation. Saima *et al.,* (2018) perform the correlation analysis between species presence/ absence data and soil chemical characteristics. Among all geo-climatic factors such as climate, altitude is of the most important one. In determining plant association within the mountain landscape as an environmental factor the altitude is important. This is because it has close correlation with temperature and down slope movement of water (Saima *et al.,* 2009, 2018; Hashim and Dasti, 2019). The distribution pattern of rock surface to soil volume is dependent on distribution of soil moisture contents in down slope (Hashim and Dasti, 2019). Various ecological factors determine the species composition, diversity, richness, and functional trait variation between species of temperate forest (Pethy and Gaston, 2006). One of these is altitude which is the most important factor that influence strongly on the vegetation structure, composition, diversity and richness (Shaheen *et al.,* 2011; Malik, 2014; 2016). Plant species in alpine mountains habitats shows strong relationship with various ecological factors, but the anthropogenic impacts of

local people disturbance cannot be ignored. Soil development in alpine ecosystem is accompanied by various interior soil erosion and movement both parallel and upright. Such disturbance is caused by severe freezing and defrosting of saturated soils, which results in diverse kinds of patterned ground. Abrupt discontinuous variations in soil edaphic conditions and at small scale heterogeneity in these factors are common features of Hilly areas. The plant species site specification in term of edaphic condition is well known (Ahmad *et al.,* 2010; Khan *et al.,* 2013). Species diversity at local scale may rise as the intercalation of such areas and their edaphic distinctness reaches to maximum (Ashton, 1969). The information about edaphic factors which effect on plant species distribution is poor where the gradient is less extreme. The studies about tropical and sub-tropical forests have shown that the distributions of the species generally associated with edaphic conditions (Poulson, 1996). The species distributions vary with topographic position.

Modern knowledge of the relationship between vegetation and environment in the diverse forest types is based on descriptions of plant species. Comprehensive species composition and its relationship to the geo-climatic factors and topographic variations are poorly known. The current investigation is a part to completing the overall vegetation picture of these areas. Recent syn-ecological procedures have developed techniques is used to set the ordination and classification of floristic data and then relating the results to environmental information and these methods are used at local as well as regional level, because this method is simpler and more advance than orthodox statistical analyses, requiring much fewer simple data and decrease the complexity of field data (Shaheen *et al.,* 2016; Saima *et al.,* 2018). Many insights of natural ecological communities have been provided by direct gradient analyses. Gradient analysis in wet temperate forests can be mostly challenging, in which large number of species are found, local taxonomic treatments was regular absence, and it is very difficult to access the diagnostic parts found in many canopy trees, epiphytes and vines, these studies have in particular rewarding because these give a large number of species and inter specific comparison of analysis. In the present study area, vegetation dynamics have been neglected by different ecologists because of the remoteness of mountains and inaccessible tracks. Little information is available for community structure and distribution patterns.

1.1. Objectives:

- Distribution of Vascular plant species was analyzed to determine if plant species occurred in discrete communities or if vegetation was continuous along the altitudinal gradient (2400-2700 m above the sea level).
- To determine, how do the species abundance and diversity vary with altitude.
- To explain the link between environmental factors and floristic patterns across the altitudinal gradient.

2. Materials and Methods:

2.1. Study Area:

The area under observation comprised of catchment area of glaciated lake locally called lake Saif-ul-Malook. The vegetation was studied along the altitudinal gradient located between 2500 m a.s.l to 3500 m a.s.l. and latitudes 34^{o} 52’ 37.04 N and 73^{o} 41’40.15 E longitude and represent the mountain ranges of Western Himalaya. The landscape of the area is quite heterogeneous in topography and thus has diversity of habitats. Steep to moderate mountain slopes, deeply incised valleys, several glaciated lakes, and snow caped mountain peaks form a complex system of undulating landscape. The

topographic heterogeneity has resulted in diverse ecological patches that harbor vast amount of differential plant species. The flora as whole is quite unique for western Himalaya. Khan *et al*., (2013) classified the vegetation of the area as moist alpine pasture. The vegetation is like arctic tundra i.e., trees less vegetation. A large area containing rolling alpine meadow is met at the crest of these ridges (Altaf *et al.,* 2020).

2.2. Climate:

The study area is located broadly in wet temperate forest climatic zone, with long, frozen winters and short cold summers. In early autumn, chilly winds bring temperatures down. Precipitation occurs in spring-summers and snow in autumn-winter. It is very difficult to find exact Climatic figures**.** The estimated mean value of precipitation ranges from 900 mm to 1350 mm provided by Khan, (1989). The relative humidity also varies from 40 to 86%. Mean temperature recorded for the month of January is -9°C in three years (Unpublished information from the local forest department). Mean maximum monthly temperature is 24°C (lowest) for August. November to February is the coldest months while the other months are moderate with pleasant temperature.

2.3. Vegetation sampling

The study area was surveyed prior to select the sites along the altitudinal gradient. Sites were selected on 3000-3100, 3100-3200, 3200-3300, m (a. s. l). All sites were selected keeping in mind the vegetation heterogeneity and accessibility. At each altitude two plots each 0.1 ha (500×20 m) were marked. In each plot 3-4 stands (10×10 m) were established. The vegetation data for presence/absence of each vascular species were monitored in each standby laying five quadrants of (1×1 m). A total of 39 stands were sampled. All the stands were placed systematically along the accessible tracks starting from 3000- 3300 m (a.s.l). These stands were studied during June to August 2017. The Plants specimens were identified using the flora of Pakistan (Ahmad *et al*., 2016).

2.4. Soil Sampling:

Surface-soil (depth of 5 cm) samples in each stand were collected and air dried and then passed through a 2mm sieve. From sieved soil, three sub-samples were taken and used for chemical analysis following by Richards, (1954).

2.5. Soil Analysis

The pH of soil saturation extract was prepared, and analysis was done by following Cramer, (2012). To determine the Electrical Conductivity (EC), First of all we prepared Soil saturation paste and used suction pump to obtain its extract. Then using CM-30 ET digital conductivity meter we determined Electrical conductivity of the extract (Cramer, 2012). Percent available Organic matter in soil samples was determined by equation: The given equation is used to determine the organic contents of soil (Cramer, 2012; Saima *et al.,* 2018).

$$\text{Soil organic matter} = \frac{\text{ml of K2Cr2O7}}{\text{Weight of sample (gm)}}\text{of} \times 9.698$$

The amount of nitrogen in soil was determined by Kjeldhal digestion method followed by colorimetric measurement of NH_4 by following (Cramer, 2012). The soil soluble phosphorous was

estimated by using Flame photometer, Jenway PFP-7 (Cramer, 2012). Potassium was calculated by using Jenway PFP7 flame photometer (Cramer, 2012). The available soil cations (Ca^{++}) were determined by the help of atomic absorption spectrophotometer (235-ATS, Made in USA). The directly readings were recorded. Soluble Sodium was determined by using Jenway PFP7 Photometer following by (Cramer, 2012). Titration method was used to determine the available content of chlorine in soil samples following by (Cramer, 2012).

2.6. Data Analysis:

The species presence/ absence data were used classification analysis. Relative frequency of species was calculated by following (Hashim and Dasti, 2019). A floristic data matrix of 91 species x 39 strands was processed by multivariate analysis. Species richness and diversity index was calculated every species in all sampled strand by the help of MVSP. Cluster Analysis was used to know the similarity/dissimilar among site samples by using a default option Farthest Neighboring and Pearson Coefficient. DCA was applied for ordination of the data set to confirm the results obtained from cluster analysis. Scatter of classification groups were plotted on overlays of the ordination axis to determine the compatibility of the two methods of data simplification (Khan *et al*., 2013; Bibi *et al.,* 2020). The Eigen values of the ordination axes were taken as a measure of magnitude of variation in the data set.

Analysis of variance (ANOVA) was done to determine the variations in soil chemical properties and among the plant communities identified by the normal cluster analysis. Graphs were drawn to highlight the significance of the factors shaping the different plant communities. The relationships between DCA axes 1 and 2 and soil characters were estimated using Pearson correlation by the help of MINITAB-Version 14.5).

3. Results:

3.1. Taxonomy

The present work reported plants species belonging to alpine landscape. Overall vegetation may be classified as grass-scrub distributed along the rim of a glaciated lake (Saif-ul-Malook). From the study area a total of 91 plant species belonging to 75 genera and 37 families were recorded. Angiosperms contributed a major share in the study area. Among the Angiosperms, 36 families comprised of 90 species were recorded. Among these species dicotyledonous species had the largest share (94.5%) followed by monocotyledonous species which contributed very minor share (4.4%) to the floristic riches of the area (Table1).

Table 1. Showing the percentage of Angiosperms in the study area

Group	Sub-group	Family	Genera	Species	%
Angiosperm	Monocot	1	4	4	4.4
	Dicot	35	70	86	94.5
Ferns	-	1	1	1	1
Total	-	37	75	91	99.9

Among the dicotyledonous families, Lamiaceae with 11 species was the largest family followed by Asteraceae with 09 species. Among the remaining families, Caryophyllaceae 6, Polygonaceae 5, Rosaceae 5, and Ranunculaceae had 4 species. The other dicotyledonous families contributed a very

little in the floristic composition of the study area. Among the monocotyledonous families Poaceae was prominent. Pteridophytes had only one species (*Equisetum arvensis)* while, Pinaceae and Cupraceae were altogether absent (Table 2).

Table 2. The plant families with highest number of genera and species in the study area.

Family	No. of Genera	Percentage (%)	No. of Species	Percentage (%)
Lamiaceae	9	12.00	11	12.09
Asteraceae	8	10.67	9	9.89
Caryophylaceae	5	6.67	6	6.59
Rosaceae	5	6.67	5	5.49
Polygonaceae	4	5.33	5	5.49
Ranunculaceae	4	5.33	4	4.40
Poaceae	4	5.33	4	4.40

Grasses and geophytes were very rare. The life form such as shrub herbs and grasses were recorded during field study are given in the (Table 3). The results exhibited that the dominating life form in the study area was herbaceous plants (91%) followed by shrubs (7%), trees were totally absent from the study area being alpine in climate. Among the component species 69% were perennial, 31% were annual (Table 3).

Table 3. Percentage composition of life form recorded in the study area.

Type	Total number	Perennial	%	Annual	%
Herb	83	60	65.93	23	25.27
Shrub	07	03	3.29	04	4.39
Fern	01	--	--	01	1.09

3.2. Vegetation Ecology

3.2.1. Diversity and floristic richness

Species richness, diversity, and evenness index for each sample site was estimated by Shannon's and Brillion's diversity indices. The values for species diversity and richness are given in (Table 4). The species richness and consequently the diversity index decreased with increased in elevation. The pattern of decrease was linear with altitude for both the diversity indices were recorded in sites located at low elevation, similarly minimum values were recorded in sites located at high elevation. Here high values indicate high diversity. Evenness was recorded 1.0 for each site. The results suggested a negative correlation of Pearson's diversity and species richness with altitude (Table 8).

Table 4. Diversity index and Species richness. Values are mean across each site.

Altitude (m a.s.l)	Species Richness	Shannon Index	Brillion Index
3000-3100	10.46	2.31	1.52
3100-3200	9.81	2.28	1.50
3200-3300	8.8	2.15	1.39

3.3. Vegetation Classification

Two main plant associations were recognized by the Normal Cluster Analysis. At first level of division the twelve samples belonging to low altitude (Association A, 3000 to 3100 m a.s.l) were separated from those located at high altitude (3100 – 3300 m a.s.l) by having *Strobilanthus attenuate, Phlomis bracteosa, Ajuga bracteosa* and *Impatiens sulcata* were altogether absent from the rest (Figure 1 and 2).

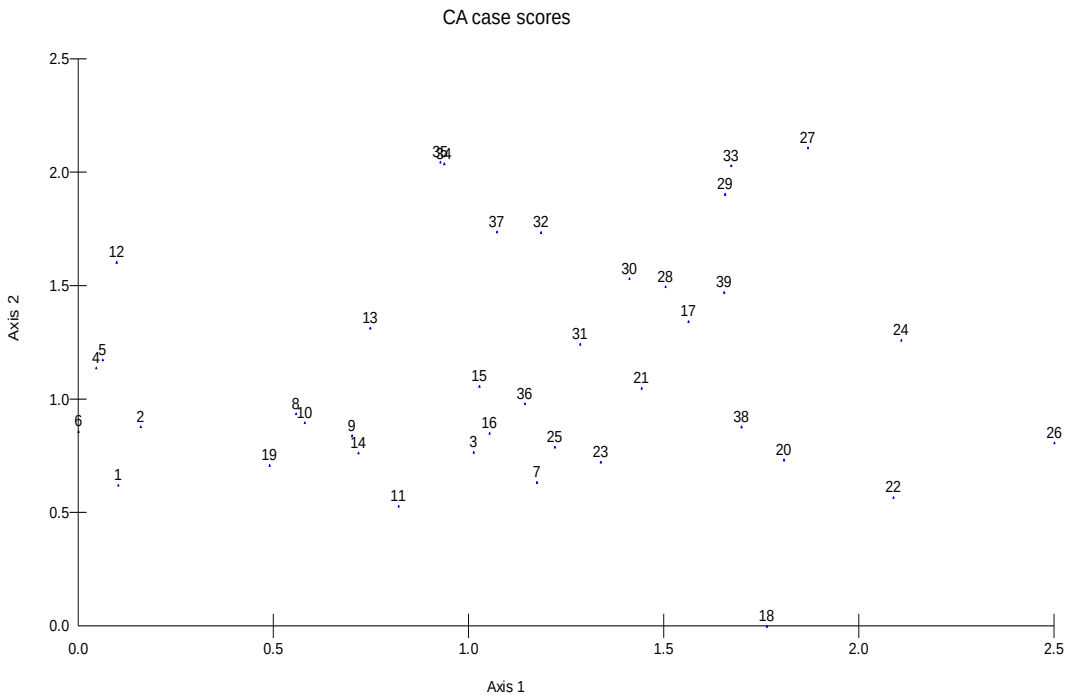

Figure 1. The Decorana (axes 1 and 2) plot of 39 samples from Naran Valley, Saif ul-malook. Stands are plotted individually, and zones are shown in which each of three associations segregated to the cluster analysis.

On second level of the division, samples belonging to the (Association B, 3100-3200 m (a.s.l) were separated from the rest by having *Stellaria media, Jasminum humile, Rosa webbiana* and *Lolium perenne* were absent from all other associations. On third level of division the stands which were located at high altitude (Association C, 3200-3300) were separated by Normal cluster analysis by having *Achillea millefolium, Cerastium cerastidies* and *Corydalis stewartii* which were altogether absent from the rest (Figure 1 and 2).

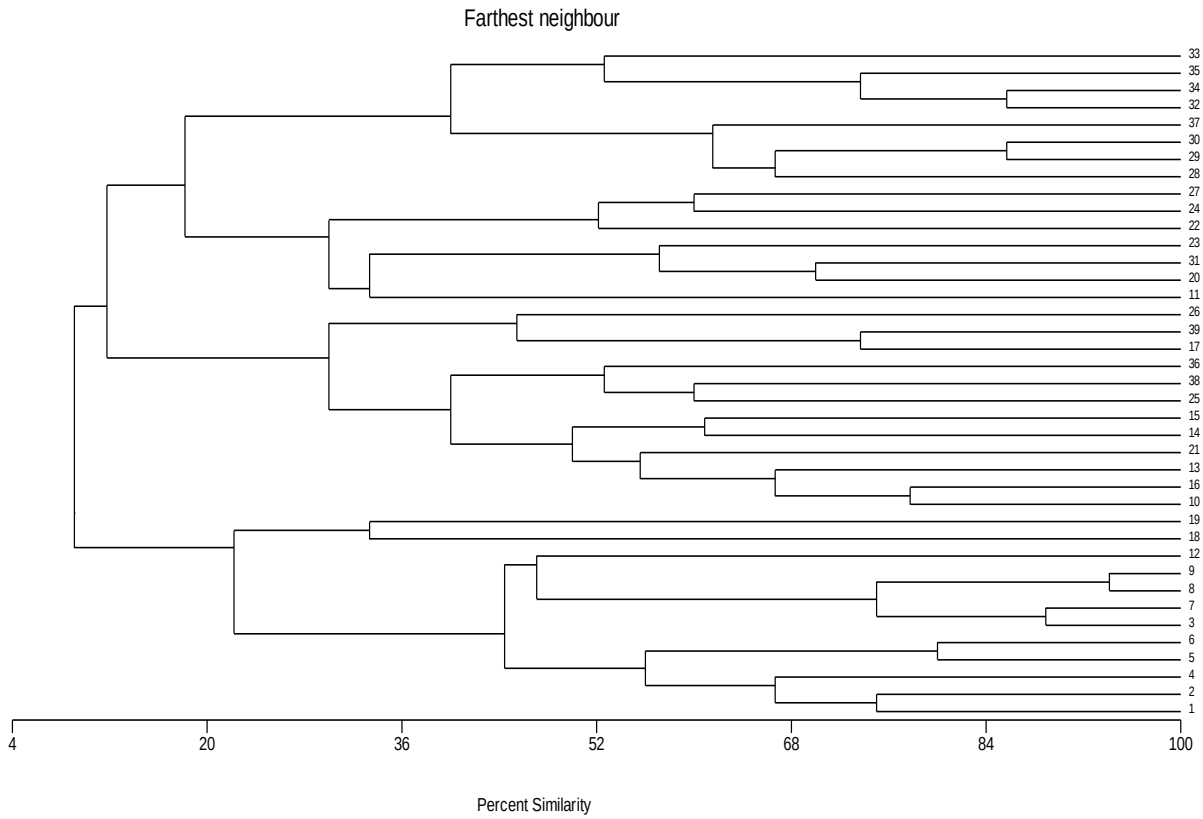

Figure 2. Dendrogram obtained from Normal Cluster Analysis for 39 samples (Quantitative Data).

These results suggested the importance of altitude in shaping the plant communities along the altitudinal gradient. The detail composition of all association is given in Table 5. Briefly description of associations presented below.

Table 5. Number and list of stands in each association identified by Normal Cluster Analysis

Sr. No	Association	Stand No.	List of Strands
1	A	13	1,2,4,5,6,8,9,10,11,12, 14,18,19
2	B	16	3,7,13,15,16,17,20,21,22, 23,24,25,26,31,36,38
3	C	10	27,28,29,30,32,33,34,35,37,39

3.3.1. Association A:

Association A was distributed at 3000 – 3100 m (a.s.l.) or slightly higher at appropriate places. This association was characterized by small sized plants which provide favorable conditions for plant survival against the high winds, low temperature, and stony rocks covered by glaciers whole of the

year. During summer season shady slopes provide the niches for most of annual herbaceous plants such as *Strobilenthus attenuata*, *Phlomis bracteosa*, *Ajuga bracteosa, Impatiens sulcata* and *Urtica dioca* which were altogether absent from other associations delineated by Normal cluster analysis (Table 6). The dominant herb species were *Poa alpine*, *Astragalus himalyansis, Trifolium repens, Potentila nepalensis, Taraxacum officinale* and *Epelobium laxum*, which mainly form the patches of green mats. *Sibbaldia cuneata, Bistarta amplexicaule, Dianthus angulate, Oxyria digyna, Sibbaldia, Aconogonon alpinum, Sporobilus, Chenopodium foliosum* are the common species and *Podophyllum emodi, Galium aparine, Anaphyllus acutifolia, Androsace rotundifolia, Capsila bursa-pastoris, Geranium wallichianum, Gnaphalium lueto-album, Myricaria germanicia, Plantago lanceolata, Polygonum amplexicule, Roselleria, Sambucus wightiana* were rare species. Among the ferns *Equisetum arvense* was also present. A few species of grass *Poa alpina* was forming small patches (Table 6). The soil of this association is slightly alkaline and characterized by having the high pH with a mean value of (7.00). The electrical conductivity value was high (3.42) as compared to other associations while Phosphorus contents was high (Table 10).

Table 6. Relative frequency of the species in each association from the Normal cluster analysis

Species name	Associations		
	A	B	C
Achillea millefolium L.	--	--	1.22
Aconogonon alpinum (All.) Schur in Verh.	2.12	1.25	1.11
Ajuga bracteosa Wall. ex Bth.	2.34	--	--
Amaranthus spinosus L.	1.43	1.11	--
Anaphalis acutifolia Hand.Mazz	0.78	0.69	--
Androsace rotundifolia Hardw.	0.78	--	--
Aquilegia pabiflora Ledebour,Mém.	--	--	2.12
Arabidopsis thaliana (L.) Heynh.	--	0.69	--
Arenaria serpyllifolia L.	1.52	--	--
Artemisia parviflora Buchanan-Hamilton	--	2.23	
Astragalus chlorostachys Lindl.	4.25	2.08	--
Astragalus himalayanus Klotzsch	--	1.15	--
Bistorta amplexicaulis (D. Don)	2.14	1.11	--
Capsella bursa- pastoris (L.) Medil	0.78	0.69	1.22
Caryophyllum reflexum Lindl.	1.22	--	--
Cerastium cerastoides (L.)	--	--	1.22
Chenopodium album L.	--	--	1.22
Chenopodium foliosum Ascherson.	1.89	1.58	--
Cirsium falconeri (Hook.f.) Petr.	1.54	1.34	--
Corydalis stewartii Fedde.	--	--	1.22
Cotoneaster microphyllus Wallich.	1.56	--	2.44
Dactylis glomerata L.	1.12	--	--
Delphinium roylei Munz	1.25	--	--
Desmodium elegans DC.	2.34	2.25	2.21
Dianthus angulatus Royle.	2.13	0.69	--
Epilobium cylindricum D.Don.	2.91	0.69	1.22
Epilobium laxum Royle.	--	1.12	
Equisetum arvense L.	0.78	--	--
Erigeron acer var. multicaulis Wall.	--	0.69	--
Euphorbia wallichii Hook.	2.67	1.08	--
Fragaria indica Andr.	2.34	1.39	1.22
Galium aparine L.	1.11	--	--

Geranium wallichianum D.Don.	--	0.69	2.44
Gnaphalium luteo-album L.	0.78	2.08	--
Heracleum candicans Wall. ex DC	0.78	0.65	--
Impatiens sulcata Wallich.	1.34	--	--
Impatiens edgeworthii Hook.	2.32	--	--
Impatiens glandulifera Royle.	--	2.13	--
Iris hookeriana Foster in Gard.	--	1.18	--
Jasminum humile L.	2.6	3.64	6.54
Myricaria germanica (L.)	--	1.67	--
Lolium perenne L.	--	1.12	--
Lotus corniculatus Linn., Sp. Pl.	1.56	--	--
Malva neglecta Wall.	1.65	--	--
Micromeria biflora Benth., Lab. Gen.	--	3.45	2.44
Myosotis palustris (L.) Nath.	0.78	--	--
Nepeta erecta Bth.	0.68	3.45	2.44
Nepeta govaniana Bth.	0.58	0.69	--
Nepeta laevigata D.Don.	--	--	2.1
Origanum vulgare L.	--	--	1.22
Oxyria digyna L. Hill.	2.33	--	--
Oxytropis glabra Ledeb.	2.13	1.34	1.22
Phlomis bracteosa Royle.ex Bth.	--	1.11	1.56
Plantago ovata Forssk.	2.45	--	--
Plantago lanceolata L.	1.34	0.69	--
Plantago major L.	0.78	1.38	--
Poa alpina L.	1.56	3.12	1.22
Podophyllum emodi Wall. ex Royle	4.01	5.43	9.32
Polygonum aviculare L.	1.22	--	--
Polygonum amplexicaule D. Don	--	1.64	2.44
Potentilla nepalensis HK.	0.78	--	--
Prunella vulgaris L.	3.13	2.22	3.66
Ranunculus diffusus D.C	--	2.08	3.66
Rosa webbiana Wall and Royl	0.67	1.11	--
Rosularia sedoides (Decne.)	--	1.34	--
Rumex nepalensis Spreng.	0.78	--	--
Salvia nubicola WallandSweet.	2.34	3.33	8.76
Sambucus wightiana Wall.ex.	1.5	--	--
Saxifraga flagellaris (Royle) Engl. and Irm.	0.72	--	--
Saxifraga jacquemontiana Dcne.	--	--	1.1
Scrophularia frutescens var. lat	--	0.69	--
Senecio chrysanthemoides D.C	--	0.69	2.44
Sibbaldia cuneata Kunze.	--	2.08	8.66
Silene indica Roxb.	2.31	3.1	2.64
Geranium nepalense Sweet, Geran.	--	--	2.44
Sporobolus sp.	2.03	1.32	--
Stachys emodi Hedge.	--	0.69	1.22
Stelerea lessertia (Wik) C.A.Mey.	--	2.78	--
Stellaria media L.	--	1.22	--
Strobilanthes attenuata Nees in DC.	2.58	--	--
Swertia paniculata Wall.	--	0.69	--
Taraxacum officinale Wigg.	3.03	8	6.2
Thalictrum falconeri Lecoyer in Bull.	2.13	1.16	2.44
Thymus linearis Benth. in Wall.	--	1.27	1.43
Trifolium repens L.	3.2	4.45	3.64

Urtica dioica L.	1.89	--	--
Valeriana pyrolifolia Decne.	2.58	1.11	1.15
Verbascum thapsus L.	--	--	1.2
Veronica anagallis-aquatica L.	--	0.21	--
Viburnum cotinifolium D.Don.	2.44	1.78	--
Viburnum grandiflorum Wall.	--	1.39	--

3.3.2. Association B:

The component species of this association were distributed at 3100 m- 3200 m (a.s.l). The flat ground and easier slopes are occupied by herbaceous pastures. The shrubs contributed least to this association including *Rubus pungens, Spiraea species* and *Viburnum cylindricum***.** Among the herbaceous strata, *Arabidopsis thaliana, Erigeron multicaulis, Saxifraga jaquemontiana, Epelobium laxum, Lolium perenne* and *Astragalus Himalayans* and *Impatiens glandulifera* were the characteristic species which were absent from all other association (Table 6). *Poa alpine, Trifolium repens Iris hookeriana, Micromeria biflora, Myosotis alpestris, Rumex nepalensis, Plantago major, Sibbaldia cuneata* and *Stellaria lessertia* were the common species in this association. S*wertia paniculata, Scrophularia latifolia, Geranium nepalensis* and *Ranunculus diffuses* were the rare species among this association (Table 6). The soil of this association showed mean value of pH 6.47 (Table 10) which showed slightly acidic properties. In this association values for soil Phosphorus contents, Potassium, Sodium, Nitrogen, Chloride and Magnesium showed the intermediate values between association A located at low altitude and the association c located at high altitude (3200- 3300 m a.s.l).

3.3.3. Association C:

This association was distributed between 3200 m and 3300 m. The divisor species were *Nepeta govaniana, Aquilegia pubiflora, Silene indica, Corydalis stewartii, Chenopodium album, Achillea millefolium, Verbascum thapsus* (Table 6). The other common herbs are *Taraxacum officinale, Iris hookeriana* and *Prunella Vulgaris.* There was a good herbaceous flora mainly between and among the shrubs. *Snecio chrysanthemoides, Poa alpine, Prunella vulgaris, Potentilla nepalensis Sibbaldia, Plantago major, Ranunculus diffuses, Rumex nepalensis* are dominant while other were least covering the field (Table 6). Soil of this association was quite poor in organic matter by having a mean of (1.048). This soil was characterized by having electrical conductivity of (2.450 ms/cm), Mg (0.16500), and a pH of (5.940) as shown in the Table 10.

3.4. Ordination

Using ordination program Indirect gradient analysis was performed for the total data set (DCA) (Braak, 1987). Rare species down weighted. Eigen values for the four axes of ordination are shown in Table 7. Axes 1 and Axes 2 were most important with Eigenvalues 0.336 and 0.136 thus DCA axis 1 and axis 2 explained most of the variations in the given data (Table 7).

Table 7. Eigenvalues and Cumulative Percentage of DCA axes 1-4

Axes	Eigenvalues	Percent of total	Cumulative percentage
1	0.336	11.19	11.19
2	0.219	7.29	18.49
3	0.205	76.80	25.30

4	0.136	4.52	29.82

Further axes, each had explained low eigenvalues and thus were ignored. The overlay of the cluster obtained from cluster analysis of sampling sites on the ordination axes I and II suggested the similarities between the two procedures of data simplification. Altitude had main impact on the floristic variation in the study area. This was certified by Pearson's Rank Correlation with the ordination score along DCA axis I. The sample scores along DCA axis 1 and altitude (Table 8) shows highly significant positive correlation *(r* = 0.944).

Table 8. Co-efficient of Pearson Correlation of factors between DCA first axis, DCA second axis, altitude, soil and environmental parameters

Parameters	Axis 1		Axis 2	
	Coefficient	Significance	Coefficient	Significance
Altitude(m)	0.613	***	0.543	***
No. of Species	-0.553	***	-0.029	NS
pH	-0.640	***	-0.588	***
E.C $mSdm^{-1}$	-0.545	***	-0.497	**
O.M (%)	-0.643	***	-0.270	*
P (ppm)	-0.397	*	-0.277	*
K (ppm)	-0.629	***	-0.549	***
Na meq/L	-0.704	***	-0.350	*
Cl meq/L	-0.597	***	-0.546	***
N (ppm)	-0.663	***	-0.553	***
Mg (ppm)	-0.517	*	-0.391	*
CO_3 (ppm)	0.261	NS	0.021	NS
HCO_3 (ppm)	-0.256	NS	0.024	NS

These results shows that the samples (low score) located at the far left hand side of the ordination diagram belongs to the associations occurring at low altitude and are characterized by having *Cotoneaster microphyllus*, *Dianthus angulatus*, *Epilobium laxum*, *Euphorbia wallichii*, *Fragaria indica, Geranium wallichianum*, *Impatiens sulcata*, *Lotus corniculatus*, *Oxyria digyna*, *Phlomis bracteosa*, *Phlomis bracteosa*, *Plantago major*, *Plantago ovate*, *Polygonum amplexicule, Sambucus wightiana, Sibbaldia cuneata*, *Silene indica*, *Taraxacum officinale*, *Thymus linearis*, *Trifolium repens* (Table 6).These species were altogether absent in samples located at the far right hand side of the diagram and representing the plant associations located at the highest altitude where *Anaphalis acutifolia*, *Gnaphalium luteo-album*, *Micromeria biflora*, *Myosotis palustris, Nepeta erecta*, *Polygonum aviculare*, *Potentilla nepalensis*, *Senecio chrysanthemoides*, *Stachys emodi* , *Verbascum Thapsus*. Site ordination reveals a marked relationship between the first axes and altitude along with soil factors (Table 8). Among the edaphic factors soil pH, organic matter, Nitrogen content, Phosphorus, Sodium concentration played the significant role in the distribution of plant species in the study area (Table 9).

Table 9: Analysis of variance (ANOVA) of different parameters

Source	DF	SS	MS	F	P
Altitude(m)	2	316092	158046	31.98	0.000
No. of Species	2	7.30	3.65	0.75	0.48
pH	2	6.1260	3.0630	32.24	0.000
E.C (mS /dm)	2	5.157	2.578	19.71	0.000

O.M (%)	2	1.5550	0.7775	11.60	0.000
N (ppm)	2	0.0037027	0.0018513	36.82	0.000
P (ppm)	2	4.63	2.31	2.31	0.114
K (ppm)	2	16561	8280	33.93	0.000
Ca (ppm)	2	8.9	4.5	0.07	0.934
Mg (ppm)	2	0.03677	0.01839	14.21	0.000
Na meq/L	2	161.73	80.87	19.69	0.000
Cl meq/L	2	108.13	54.07	27.07	0.000

3.5. Soil analysis:

Almost all the soil variables included in the present investigation were significant p> 0.05. Among the variables altitude, showed the highest F value suggesting the importance of altitudinal difference in determining the composition of plant association along the altitudinal gradient (Table 9). The number of species decreases with the decrease of altitude. At lowest altitude numbers of species were three-fold lower than at the highest altitude. The soils at the highest altitude were circum-neutral in reaction showed lowest value for Ec (Table 10). Other soil variable pH, O.M, N, K, P, Ca, Mg, CO^3, and HCO^3 showed no clear trends along the altitude gradient but exhibit significant difference among the plant's association. For example, soil of association C was poor in soil nutrients than the remaining association identified by cluster analysis (Table 10).

Table 10. Mean and Standard deviation of parameters of all association

Source	Statistic factor	A	B	C
Altitude (m)	Mean	3034.2	3158.8	3274.0
	St. Dev	52.1	91.5	39.5
No. of Species	Mean	10.250	9.824	9.100
	St. Dev	3.019	1.704	1.792
pH	Mean	7.000	6.4729	5.920
	St. Dev	0.2523	0.3757	0.2265
E.C (mS/dm)	Mean	3.4208	2.9388	2.4500
	St. Dev	0.3905	0.3765	0.2915
O.M %	Mean	1.5667	1.2353	1.0480
	St. Dev	0.3675	0.2390	0.0397
N ppm	Mean	0.055083	0.041824	0.029100
	St.Dev	0.005334	0.009632	0.001197
P ppm	Mean	10.429	9.897	9.522
	St.Dev	1.047	1.039	0.862
K ppm	Mean	283.08	253.12	228.40
	St.Dev	12.88	17.32	15.50
Ca ppm	Mean	23.134	23.001	21.969
	St.Dev	8.663	7.902	7.498
Mg ppm	Mean	0.24583	0.19824	0.16500
	St.Dev	0.04641	0.03729	0.00850
Na meq/L	Mean	12.113	8.5321	6.912
	St.Dev	2.697	2.042	0.351
Cl meq/L	Mean	11.377	9.171	6.931
	St.Dev	1.434	1.517	1.175

4. Discussion

Himalaya showed divers patterns of species distribution. The range of climatic variability along an altitudinal gradient favors several species to survive in canopied moist temperate forest. The distributional patterns of several species depend on altitudinal limits (Kharakwal *et al.,* 2005; Khan *et al.,* 2013; Hashim and Dasti, 2019; Altaf *et al.,* 2020; Bibi *et al.,* 2020). Altaf (2020) described that species site preference, which determines the diversity and richness patterns along an elevational gradient in mountain temperate forest. Pausas and Austin, (2001) also reported that the distribution of species richness is mostly encountered by environmental factors. Kumar and Ram, (2005) described that species richness and diversity decreased across the elevation gradient in Western Himalaya. Our research work revealed that maximum species richness was at lower elevation, as compared to higher elevational forests as reported by Gairola, (2009) and Khan *et al.* (2013). Gairola, (2009) has reported that maximum species richness and diversity in the *Pinus roxburghii* forests located in low elevation having maximum rainfall, minimum snowfall, and low wind speed velocity. Singh *et al.* (1994) reported the highest species richness and diversity in the lowest forest located in Western Himalaya which receives the maximum rainfall in monsoon season. Austin *et al.,* (1996) had explained that species richness was greatest at lower elevation and warmer sites. The overall pattern of species richness showed a sharp decline as the altitude increased beyond 3000 m (a.s.l). Similar distribution pattern of species richness in alpine area was reported by Rawal *et al.* (1991). This pattern of decrease in species richness with altitude was also reported by Rahbek, (1997). The present investigations suggested uni-model pattern with decreasing species richness along increasing elevational pattern. These results are consistent with the finding of Terborgh, (19971) and Khan *et al.* (2011). But contradict the observation of Rahbek, 1995, who suggested the lack of mid elevation peak. The low species richness result depicted in figure show that species richness in association-C may be due to slow uptake of nutrients from the soil, decrease nutrients availability, increase soil leaching due to runoff, and increase soil organic matter by Taner *et al.* (1998). Opposite trends in species richness are expected in base rich association A, where abiotic factors are not limiting factors. The staggered elevation distribution of the divisor or dominant species showed that dominants are likely to be each other's most important competitors and important determinants of each other's distributions (Whittaker *et al.,* 1983). The tendency of certain plant families such as Lamiaceae, Caryophylaceae, Polygonaceae and Rosaceae are the species rich about the entire elevation gradient (Gentry, 1988). The results obtained from the current investigation showed a total of 91 plant species belonging to 75 genera and 37 families from 49 sampling sites. The dominant families were Lamiaceae, Asteraceae, Caryophylaceae, Rosaceae, and Polygonaceae. Theophytes (Annual herbs) constituted 23 species followed by Chemophytes (Perennial herbs) of 60 species and Perennial shrubs 3 species. Tree strata were completely absent. These distributions may range from anthropogenic to basic geomorphological process of surface movement through the action of wind, splashing and snow melting glaciations. These factors may be contemporary or historical and range from the sweeping climatic changes to current harvesting activities by local villagers and grazer who may remove a woody species from wide area for use as fuel.

The three-association produced by the Cluster Analysis were confirmed by DCA. The two procedure of data interpretation expressed very similar results. We take thus results to confirm the partially overlapping nature of association in this part define by ordination axis. The axis scores represent the major environmental influences which affect the distribution patterns of plant communities. The first ordination axis represents a gradient from low altitude to high altitude from left to the right hand in the diagram. The samples belonging to the lowest elevation are found on the extreme left-hand side of the diagram while the samples from high altitudes tend to concentrate on the right side of the ordination. Besides the altitude the distribution of the species is potentially affected by

soil properties (Dasti *et al.*, 2007; Wazir *et al.,* 2008; Saima *et al.,* 2009). The correlation analysis with DCA scores confirms that there is a significant relationship between soil properties and community type. The DCA results exhibited that elevation gradient, soil pH, EC, soil organic matter, Potassium, Sodium, Chlorine, Nitrogen and Mg are the significant factors for the distribution and assemblage of plant species. The rainfall and redistribution of rainfall water along with elevation gradient is important ecological factor. The run-off generated by the higher altitude will then move downward (run-on) and create moisture gradient along which vegetation change occur. Therefore, the downslope movement of water plays an important role in determining the zonation along the altitudinal gradient. Thus, the first ordination axis represents a moisture gradient from upper rocky zone (with low score Association A), lower zone (with high score, Association B and C). Vegetation of the upper zone consisted of *Aquilegia pubiflora*, *Achillea millefolium, Silene indica, Verbascum thapsus* , *Chenopodium album*, *Cerastium cerastiodies,Corydais stewartii, Nepeta govaniana*, *Nepeta laevigata* , *Saxifraga flagellaris, Silene indica* and Lower zone *Ajuga bracteosa, Areneria serpylliforia,, Androsace rotundifolia, Caryophyllum reflexicum, Delphinium roylei*, *Dactylis glomerata, Equisetum arvense, Galium aparine, Heracleum candicans, Impatiens sulcata, Myricaria germanicia, Lotus corniculatus, Malva neglecta*, *Origanum vulgare, Phlomis bracteosa*, *Podophyllum emodi* , *Polygonum amplexicule*, *Rosularia sedoides*, *Salvia niobicola*, *Sambucus wightiana*, *Strobilanthes attenuata*, *Urtica dioica* So it suggested that most of the variation in the plant assemblage pattern is affected by altitude and soil chemistry (Wazir *et al.*, 2008).

Beside the altitude, soil chemical characteristics play significant role in community composition and plant assemblage. The species belonging to association A occur on slightly alkaline pH. The decreasing trend in pH with increase in altitude is because of downward movement of nutrients that affect the shaping of plant communities. These results similar to the finding of Pendry, (1994); Pendry and Proctor, (1996) but contradict the results of Veneklaas, (1991) who reported highly alkaline pH of soil at in forest distributed at high elevation than the soil in nearby the forest located at low elevation. Soil organic matter increase with altitude partly because of a reduce decomposition rate at low temperature but often due to temporary waterlog conditions created by melting snow at upper mountains. The available soluble phosphorus is low at in association located at higher altitude. The elevational decreases in phosphorus may be due to high concentration of organic matter which reduce potential of phosphorus fixation (Tanner *et al*., 1988).

5. Conclusion

The correlation analysis of axis scores with soil cations and anions suggested that vegetation is a complex collection of substrates, specialist, and generalists. It may be concluded that both classification and ordination are able to delimit the plant associations according to their environments. Topographic heterogeneity is an important factor that play important role in the community composition.

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Small Scale Altitudinal Variation in Gap Vegetation in Temperate Forest: A Case Study in Mukhshpuri, Ayubia National Park, Pakistan

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Abstract

The current knowledge of the relationship between vegetation and environment in Himalayan conifer forests of Pakistan is based on subjective descriptions of ecological conditions from field experience and interpretation. Wet temperate forests of Pakistan are interesting because at suitable elevations it merges downward with the tropical forests and upward with the alpine meadows. Vegetation from 57 stands (10 x 10 m) established between 2500 - 2800 m (a.s.l.) along an elevation transect were sampled in the study area under consideration. Soil was analyzed for their chemical properties. The presence absence data of all the species from each sampling stand were clustered with hierarchical method of classification. The stands were also be subjected to Detrended Correspondence Analysis (DCA) using the DECORANA package. The relationships between soil characters, altitude and other unmeasured environmental factors were determined using Spearman's rank correlation. It is concluded that elevation play an important role in species distribution and richness and other soil parameters also show variation along altitude. As altitude increases organic matter, electrical conductivity, calcium, potassium, magnesium, chloride, carbonates and bicarbonates decreases and show adverse effect on species diversity and richness.

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1. Introduction:

Temperate forests are the highly species rich and structurally complex plants communities on the earth (Vazquez and Givnish, 1998). Recently, numerous descriptive studies have focused on the trends in the diversity, composition and structure of moist temperate forests along diverse environmental gradients including patterns of rainfall, topographic and edaphic heterogeneity (Dui-Venvoorden, 1996; Saima *et al.*, 2018; Hashim and Dasti, 2019; Altaf *et al.,* 2020; BiBi *et al.,* 2020; Nazir *et al.,* 2020). Several detailed studies have examined such community attributes along substantial elevation gradients (Liberman *et al.,* 1996), but limited number of ecologists worked on effect of altitudes on vegetation distribution, and no one have investigated the variations in community distribution in term of long transect along the ecological gradients in forest gaps. Moist temperate forests located in Western Himalayan region of Pakistan are situated at higher elevation ranging from (1800) m (a.s.l.) to 3000 m (a.s.l.) has been severely disturbed by deforestation, grazing and anthropogenic activities in past (Khan *et al*., 2011; Hashim and Dasti *et al.,* 2019). Most recently it has been affected by constructions and agricultural practices. In contrast, some areas of lower western Himalayan Mountains still provide rare opportunities for studies of comparatively undisturbed vegetation, particularly in forest gaps. Mainly the tightly reserve forest is suitable for such studies.

Because of difficult accessibility and severances of climate, most of valleys still virgin for ecological studies (Liberman *et al.,* 1995). Hence creates attractiveness for modern ecologist to make studies in such mountainous landscape and diverse plants niches. These mountainous regions are farther from air pollution sources. After receiving monsoon rainfall variety of flora appears for a short period and contribute specific type of vegetation (Richard, 1996). The patterns of distribution of vegetation across the elevation gradient in these moist temperate forests are monitored by several ecological factors. Altitudinally defined climatic and soil factors are considered to be chief determinants of variations in species composition and community structure (Whittaker, 1975). The forest located at lower slope harbor more diverse species than that of located at higher elevation. At high mountain systems, plant species diversity decreases with increasing altitude because of decreasing trends temperature regime (Hashim and Dasti, 2019).

Recently, Rahbek (1997) demonstrated that in Himalayan temperate forest species show mid elevation peak in distribution pattern along elevation gradient. This is because of high intra specific competition among the species for limited resources such as low forest gap reduces availability light for active photosynthesis by ground vegetation that ultimately resulted lower species richness and diversity at lower elevation similarly at high elevation species richness and diversity decreases as a result of harshness of climate, lower temperature and high wind speed and snow pressure reduces the diversity (Austrheim, 2002; Marrs *et al.,* 1988). Soethe *et al.,* (2008) described that availability of soil nutrients and water resources through reallocation of rainfall water by runoff is governed by elevation gradient. The distribution patterns of runoff limit the variations in vegetation type and affect the plant assemblage (Wazir *et al*., 2008; Dasti *et al.,* 2007).

Concave and convex landscape terrain is also significant aspect in receiving the redistribution of rainfall water and major cause of zonation and distribution pattern of plants group. The accumulation of run-off takes place at numerous scales from small depression to large paddles resulted in formation of niches and habitats of various kinds and sizes (Orshan, 1986.) Variations in the species diversity along elevational gradient have been the topic of numerous ecological studies (Vazquez and Givnish, 1998). Most of them found a hump shaped distribution, showing peak species diversity near

the middle of gradient (Zhang and Ru, 2010). The plant community structure and distribution pattern of Himalayan Forest are poorly understood (Peer *et al.,* 2007). Western Himalaya not only supports huge floristic diversity (Sharma *et al.,* 2010), but also store large carbon stocks (Dar and Sundarapandian, 2015).

Ashton (1969) described that plant species diversity is encountered by edaphic variations, as edaphic differences increase the plants diversity also increased. Wazir *et al*. (2008) had described that in high elevated mountains vegetation patterns also depends on availability of soil water and redistribution of rainwater. Beside the importance of redistribution pattern and availability of rainwater soil chemical properties also play important role in distribution pattern and dominance of plants species at different elevations. Silver *et al.* (1994) and Cramer, (2012) presented that soil chemical reactions (pH) had significant influence on distribution patterns of plant species. It is thought that floristic composition is often linked with soil chemical reaction (pH). Lower values of soil pH discourage the species richness at high elevation and slightly high pH support the high species richness (Saima *et al*., 2018).

Soil cations exchange capacities have great influence on phytodiversity. Variation in soil characteristic in term of soil pH, soil moisture availability, cations and anions concentration are conjoint attributes of mountainous landscapes (Hall and Swain, 1976). These discontinuities in edaphic conditions& edaphic factors are important in shaping plant communities. Cramer, (2012) described that with the increase in elevation Soil cations and anions decreases, that affect the type of vegetation, but comparable data is not available from temperate forests of Pakistan and particularly in study area because studies on Himalayan mountains of Pakistan have mainly been floristical (Richards,1954; Grubb, 1976; 1977) diagnosis and improvement of saline and alkaline soils. Recently numbers of ecologist have used numerical approaches for the classification and determination of limits of plant groups across the elevation gradient (Ahmad, 1989; Saima *et al.,* 2009; Khan *et al*., 2011; Shaheen *et al*., 2011). These methods are useful in determining the relationship between the geo-climatic factors and formation of plants groups along elevation gradient.

1.1. Objectives:

- To find out relationships between soil characters, altitude, and other unmeasured environmental factors.
- To determine, how do the species abundance and diversity vary with altitude.
- To determining the relationship between the geo-climatic factors and formation of plants groups along elevation gradient.

2. Materials and Methods:

2.1. Study Area:

The study area is located in one of the temperate forests in Mukshpuri Ayubia National Park and situated 90 km North of Islamabad- the capital city of Pakistan. These reserve forests are the largest protected areas of the country. Overall, human disturbances and clearing are currently sparse, and most of the forest is undisturbed. The vegetation in the study area corresponds to the Sino-Japanese phytogeographical region. Physiognomically it consists of thick canopy forestland in which conifers i.e. Pinus, Cedrus and Abies are the dominant taxa. The herbaceous vegetation appears during summer months and persists as green forage for 3-5 months (till late September). The flora is highly diverse mostly composed of annual forbs.

2.2. Climate:

The overall climate of the area is moist temperate continental type. However high peaks remained covered with snow in most part of the year. Whole the year, study area faces long winters and short summers. Most of the rainfall occurs during summer. Winter showers are infrequent and mostly occur in the form of snowflake. The annual mean precipitation at Mukhshpuri is 120 mm and the maximum amount occurs during the winter rainy season (July and August). The minimum humidity in percentage is recorded in June which is 50% and highest level is in the month of August which is 83%, in this case the average humidity of the year is 65%. The annual mean temperature at Mukhshpuri is 11.41^{o}C. The maximum temperature prevails in the month of June and July and the minimum temperature in the months of January and February.

2.3. Vegetation sampling

After repeated survey of the area, sampling sites were selected along the accessible terrain with the goal of covering the range of altitudinal heterogeneity and with a diverse ground vegetation and floristic (Figure 3). Vegetation sampling was carried out at 2500-2800 m (a.s.l.). All the sampling sites were along the accessible elevation depending upon the topographic level of the sampling sites. At each elevation 5-10 stands, 10×10 m each were laid systematically along the altitudinal transect. In each quadrat all the vascular plants were recorded including fern species. The altitudinal profile of the sampling unit was measured using a calibrated altimeter. The presence/absence data were collected of the species in the study area. A total of 57 samples were obtained containing 70 species. Plants were identified according to the flora of Pakistan. Samples of surface-soil were collected and were dried in the field and returned to lab for analysis following Richards, (1954).

2.4. Vegetation and Analysis

2.4.1. Plant Species Diversity and Vegetation Classification

The presence/absence data of 70 species found in 57 stands were used to determine the community composition through clustering analysis using Multivariate analysis (Version 3.2). Soil chemical reaction (pH) was recorded by using digital pH meter Richard, (1954). Soil EC was determined by using CM-30 ET digital electrical conductivity meter. Total soil organic matter contents were obtained by Walkely and Black's method (Nelson and Sommers 1982). The total of dissolved nitrogen content in soil was determined by Jeldhal method (Saima *et al.,* 2018). The amount of soluble anions in the soil was determined by spectro-photometer (Richard, 1954). Flame Photometer (Jenway-PFP7) was used for the determination of soluble sodium/potassium. The total content of soluble phosphorous in the soil was determined spectro-photometer (Oslon *et al.,* 1954). Total available Chloride (ppm) content was measured by the electrometric method was used as described by senior author Arao Itano & Akira Matstuura (2014). Dissolved Carbonates and Bicarbonates were determined by pH-titration Method by following Richard, (1954).

2.4.2. Soil and Vegetation Correlation

To determine the relative importance of soil characteristics in the formation of communities along the elevation gradient used the analysis of variance (ANOVA with help of MINITAB version 3.2

statistical software). The interaction between soil characters and DCA axes 1 and 2 were determined using Pearson correlation analysis.

3. Results:

3.1. Diversity and Floristic Richness

Diversity was estimated by species richness, Shannon's diversity for species and the Evenness index for each sample site. The values of diversity range from 7.86 to 11.13. High values indicate high diversity (Table 1). Similarly, the values estimated for evenness range suggested high evenness in the study area. Diversity index and species richness exhibited a strong negative correlation (*P<0.05, **P<0.01, ***P<0.001) with altitude (Table 1). Results depicted in Table 6 shows that lower the elevation higher the diversity and species richness.

Table 1. Shannon Diversity Index Evenness and Species Richness among five associations

Associations	Index	Evenness	Species richness
A	2.17	1	9.07
B	2.29	1	9.29
C	2.41	1	11.33
D	2.03	1	7.86
E	2.15	1	9.8

3.2. Classification

3.2.1. Normal Cluster Analysis

Among Five plant associations were recognized by the Normal Cluster Analysis (Figure 5). These associations were not arbitrary but related to edaphic and altitudinal conditions. The composition of stands, comparative floristic richness with taxonomic diversity along the altitude and divisor species of each association is given in the Table 2.

Table 2: Association and their divisor species with number of stands identified by Normal Cluster.

Associations	Stand No.	List of Stands and Divisor /Dominant species
A 2500-2560m	15	14,15,21,22,23,24,26,27,28,30,37,38,40,41,42
		P. multiflorum, F. indica, I. bicolor, S. urticifolia, S. urticifolia, G. wallichianum
B 2560-2620m	7	3,16,17,19,20,25,29
		A. venustum, O. contiguum, R. sceleratus, S. nubicola, V. canescens, W. amherstiana
C 2620-2680m	6	2,9,10,11,12,55
		C. sativa,P. major, P. lanceolata, S. chrysanthemoides, P. vulgaris
D 2680-2740m	14	18,31,32,33,34,36,43,46,47,48,49,50,52,54
		C. nutans,C. nubigena, M. biflora, P. alpine, C. affins, F. indica, P. nepalensis, T. repens
E 2740-2800m	15	1,4,5,6,7,8,13,35,39,44,45,51,53,56,57
		C. stewartii, C. affinis, R. nepalensis, T. repens

3.2.1.1. Association A

This association was distributed at about 2500-2560m and characterized by herbaceous plants such as *F. indica, I. bicolor, Scrophularia, urticifolia* and *G. wallichianum* which were dominant in this association*. P. multiflorum* was divisor species of this association and was absent altogether from other associations separated by Normal cluster analysis (Table 3). *C.bursa-pastoris, E. angustifolium, P. lanceolata, P. vulgaris, S. nubicola* and *T. emodi* were rare species and commonly found on the top of slopes at high elevation. The soil of this association was rich in pH (7) contain nitrogen, magnesium, chloride and carbonates. But other soil parameters like potassium are intermediate in percentage. Altitude is intermediate than any other delineated by normal cluster analysis. Soil of this association is neutral and moves towards slightly alkaline.

3.2.1.2. Association B

This association was distributed at about 2560-2620m. This association consist of 45 species belong to 43 genera. The association was characterized by annual herbs. The dominant species of this association were *A. venustum, O. contiguum, R. sceleratus, S. nubicola, V. canescens* and *W. amherstiana*. The common shrubs of the association were *C. affins, R. webbiana, R. fruticosus, S. daphnoides, S. tomentosa, P. jacquemontiana* and *S. urticifolia.* The common fern was *D. ramosa. C. Montana* and *H. nepalensis* were climber species among this association. This association contains high nutrients percentage like potassium (1.6786) and bicarbonates (6) but only chloride ions was low percentage. Some other soil parameters were present in intermediate in percentage like PH, organic matter, nitrogen, carbonates and magnesium.

3.2.1.3. Association C

This association leads to altitude of 2620 to 2680m. This association was characterized by having divisor species *C. sativa* which was altogether absent from rest of the association recognized by Normal Cluster Analysis. The most abundant plants among tree strata found in this association were *A. pindrow* and *P. wallichianum.* Among shrubs in this association *V. grandiflorum* and C. *affins* were dominating in this association (Table 3). The climber species was *H. nepalensis*. This association was characterized by more bicarbonates, magnesium, nitrogen and potassium as compared to association D and E. The soil of this association was acidic with (6) pH. Other soil parameters such as carbonates and organic matters were low in percentage as compared to association D and E.

3.2.1.4. Association D

This association was situated from the altitude of 2680m to 2740m. This association was also dominated by herbaceous plants named as *F. indica, M. biflora, P. alpine, P. nepalensis and T. repens. C. nutans* was the characteristic species of this association (Table 3). Among these species *E. angustifolium, E. wallichii, H. maximum, I. chrysantha, M. alpestris, N. govaniana and V. canescens* were rare species (Table 3)*. V. grandiflorum*, *R. fruticosus* and *S. urticifolia* contribute major share among shrub strata of this association. *J. humile* was climber species of this association. More nitrogen, potassium, chloride, magnesium, carbonates, and bicarbonates as compared to association E were observed. But fewer amounts of pH and organic matters as compared to E. Soil of this association were acidic in nature. Altitude of this association is 2699.6m.

3.2.1.5. Association E

This association was distributed from 2740 to 2800m. This association was characterized annual herbs which appeared in summer after snow melts on mountains rocky slopes and disappear in winter seasons mainly consists of *T. repens, R. nepalensis, P. major, G. wallichianum, I. bicolor, P. vulgaris* and *M. biflora.* Among these species *C. stewartii* was characteristic species and confined only to this association. *B. falcatum, C. japonica, F. indica, G. boreale, M. alpestris, N. erecta, P. lanceolata, S. nubicola, S. emodi, T. serphyllum* and *T. pratense* contribute a minor share in community composition delineated by normal cluster analysis (Table 3). *T. emodi, V. malissifolia* and *V. canescens* were rare species. Forest open canopy gaps were dominated by common perennial shrubs *C. montana, S. daphnoides, S. saligns, S. tomentosa, S. urticifolia* and *V. grandiflorum*. Among ferns *A. venustum* and *O. contiguum* were found. Among the conifers *A. pindrow* showed the major share. In this association following soil parameters gradually decrease from association A to E such as nitrogen, potassium, carbonates and bicarbonates. Soil also goes to acidic in nature.

Table 3. Relative frequency of the species in each association from the normal cluster analysis, arranged in ascending order of DCA score, axes 1 and 2.

Species name	Associations				
	A	B	C	D	E
A. pindrow	--	--	2.94	--	0.68
A. millefolium	--	1.20	--	--	1.36
A. venustum	3.68	6.02	--	--	0.68
A. petiolata	2.94	2.41	--	1.82	--
A. wallichianum	2.94	1.20	--	3.64	2.04
A. falconeri	--	1.20	--	--	1.36
B. perennis	--	1.20	--	--	1.36
B. falcatum	--	1.20	1.47	--	0.68
C. sativa	--	--	4.41	--	--
C.bursa-pastoris	0.74	--	1.47	--	1.36
C. nutans	--	--	--	3.64	--
C. nubigena	2.94	--	2.94	10.00	3.40
C. leucanthmum	--	--	--	--	2.04
C. Montana	--	2.41	--	--	1.36
C. japonica	--	1.20	1.47	--	0.68
C. stewartii	--	--	--	--	2.04
C. affins	2.21	1.20	4.41	5.45	6.12
D. ramose	2.94	2.41	--	0.91	--
E. angustifolium	0.74	1.20	--	0.91	--
E. wallichii	1.47	--	2.94	0.91	1.36
F. indica	7.35	3.61	--	8.18	0.68
G. boreale	--	1.20	1.47	--	0.68
G. pusillum	--	1.20	--	--	1.36

Species name	Associations				
	A	B	C	D	E
G. wallichianum	3.68	2.41	1.47	1.82	3.40
H. nepalensis	--	1.20	1.47	--	0.68
H. maximum	1.47	--	--	0.91	0.68
I. bicolor	3.68	2.41	--	0.91	2.04
J. humile	0.74	--	--	1.82	--
L. cachemiriana	--	1.20	--	--	2.04
Leonurus cardiac	0.74	1.20	1.47	--	--
M. neglecta	--	2.41	2.94	--	--
M. biflora	1.47	1.20	5.88	6.36	4.76
M. alprstris	2.94	1.20	2.94	0.91	0.68
N. erecta	--	1.20	1.47	--	0.68
N. govaniana	1.47	--	--	0.91	--
O. contiguum	4.41	7.23	1.47	--	2.04
O. vulgare	1.47	1.20	--	--	--
P. emodi	2.21	--	1.47	--	--
P. jacquemontiana	--	1.20	--	--	1.36
P. wallichiana	0.74	1.20	1.47	--	--
P. lanceolata	0.74	--	5.88	--	0.68
P. major	2.21	--	8.82	1.82	4.76
P. alpine	4.41	4.82	2.94	10.00	2.04
P. multiflorum	2.21	--	--	--	--
P. nepalensis	1.47	--	2.94	3.64	2.72
P. geradiana	--	1.20	--	--	1.36
P. nepalensis	--	--	--	5.45	1.36
P. vulgaris	0.74	--	5.88	3.64	4.08
R. sceleratus	4.41	4.82	1.47	1.82	--
R. macrophylla	1.47	--	--	--	0.68
R. webbiana	--	1.20	--	--	1.36
R. fruticosus	0.74	1.20	--	2.73	--
R. nepalensis	2.21	--	1.47	1.82	6.12
S. daphnoides	--	1.20	--	--	1.36
S. nubicola	0.74	4.82	--	--	0.68
S. saligna	--	--	--	--	2.04
S. urticfolia	3.68	3.61	--	1.82	1.36
S. chrysanthemoides	1.47	2.41	7.35	3.64	2.04
S. tomentosa	--	1.20	--	--	1.36
S. emodi	2.21	2.41	1.47	--	0.68
S. urticifolia	4.41	2.41	--	0.91	2.04
T. serphyllum	--	1.20	1.47	--	0.68

Species name	Associations				
	A	B	C	D	E
T. pretense	--	1.20	1.47	--	0.68
T. repens	1.47	2.41	4.41	5.45	6.80
T. emodi	0.74	--	2.94	1.82	0.68
U. dioca	--	--	1.47	--	1.36
V. malisaefolia	1.47	--	--	--	0.68
V. grandiflorum	4.41	--	4.41	3.64	4.08
V. canescens	3.68	7.23	--	0.91	0.68
W. amherstiana	2.21	2.41	--	1.82	--

3.3. Gradient Analysis

The axis-1 and axis-2 accounted for 0.504 and 0.316 correspondingly of the total unevenness in composition of species, while 3rd and 4th axis have only 0.243 and 0.212 respectively (Table 4). This shows that first two axis when taken together can be considered as practically high-quality characterization of spatial distribution of species.

Table 4. Eigenvalues and Cumulative Percentage of DCA axes 1-4

Axes	Eigenvalues	Percent of total	Cumulative percentage
1	0.504	9.146	9.146
2	0.316	5.733	14.879
3	0.243	4.411	19.289
4	0.212	3.852	23.142

The same five groups were evident when the five clusters produced by multivariate analysis were plotted on the first two axis were scattered diagram (Figure 1). The results depicted (Figure 1) revealed the gradual change in community composition with partially overlapping fashion along the spatial gradient from high altitude to lower altitude.

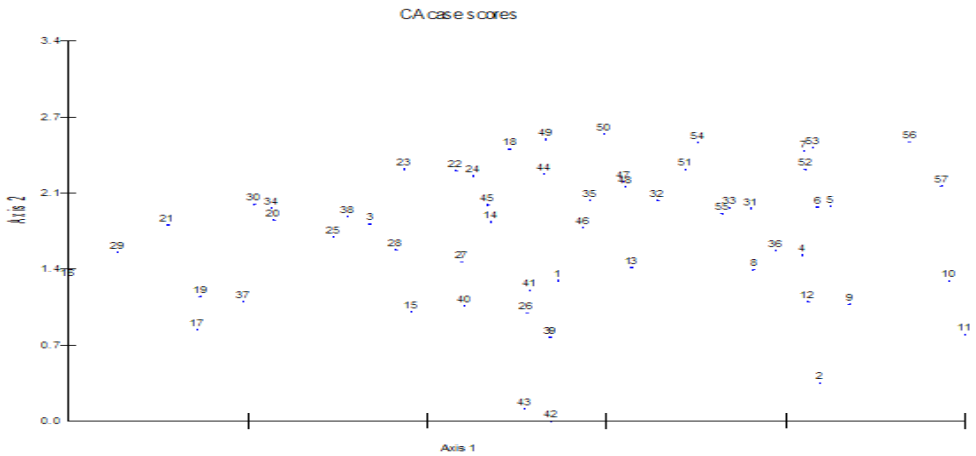

Figure 5: Ordination of plant species along DCA axis 1 and 2 among 57 stands.

This pattern of site and species distribution suggested considerable differences in habitat along the altitudinal gradient. The plants groups delineated by the Cluster Analysis and confirmed by the ordination were not arbitrary but rather dictated by several edaphic factors. The physical and chemical soil data shown most of edaphic variables in present investigation showed significant differences among these groups. Soil pH, organic matter, potassium, magnesium and bicarbonates appeared to be the most important parameters in determining the differences in the substrata of plants groups and showed high F-Values (ANOVA). The relationship between environmental variables and vegetation composition may be assessing by examining their correlation with DCA axis along the vegetation ordination (Table 5).

Table 5. Spearman Rank Correlation Coefficient of Qualitative Data, Detrended Correspondence Ordination axis Scores and Soil parameters for Ayubia National Park.

Soil parameters	Axis 1	Axis 2
Altitude (m)	0.340 0.010	0.171 0.204
Electrical conductivity (ds/cm)	-0.138 0.308	-0.238 0.075
Soil Reaction (pH)	-0.460 0.000	-0.144 0.286
Organic matter (%)	0.021 0.875	0.093 0.492
Nitrogen (%)	-0.070 0.607	-0.052 0.699
Phosphorus (ppm)	-0.012 0.928	-0.251 0.059
Potassium (ppm)	0.076 0.576	-0.313 0.018
Calcium (ppm)	-0.067 0.618	-0.228 0.088
Magnesium (ppm)	0.021 0.876	-0.318 0.016

Sodium (ppm)	0.075 0.676	-0.328 0.013
Chloride (ppm)	-0.213 0.0378	0.112 0.004
Soil parameters	Axis 1	Axis 2
Carbonates (meq/liter)	-0.054 0.692	-0.826 0.000
Bicarbonates(meq/liter)	0.025	-0.265

Examine the relationship between environment variable and stand score along DCA axis 1 and 2. The results recommended the composition of the feeble flora is given by large number of soil parameters. However, their relative significance is complex to conclude. In the present investigation altitude has the overriding importance in determining the species composition. The species from the high altitude were located at the left hand side such as *C. nutans, C. nubigena, E. wallichii, G. boreale, H. maximum, J. humile, P. major, P. lanceolata, P. nepalensis, P. nepalensis, P. vulgaris, R. nepalensis, R. webbiana, T. repens, T. pratense, T. emodi, V. grandiflorum* while the species from low altitude were located on the other side of the ordination diagram such as *A. millefolium, Allaria petiolata, A. wallichianum C. japonica, C. affinis, D. ramosa, E. angustifolium, F. indica, G. wallichianum, I. bicolor, L.cachemiriana, M. biflora, M. alpestris, N. govaniana, R. sceleratus, R. fruticosus, S. urticfolia, S. chrysanthemoides, S. tomentosa, S. urticifolia, T. serphyllum, V. canescens* and *W. amherstiana.* The soil pH showed the strongest correlation with DCA axis 1 (Table 5). While the negative relationship between DCA axis 1 & pH suggested that pH decreases as one move from low altitude to high altitude (Table 5). The soils of low altitude are characterized by having high values of pH, nitrogen, Potassium, magnesium, chloride, carbonates and bicarbonates (Table 5).

4. Discussion

The conifers generally form a complete forest and there is relatively little admixture with broad leaved trees. Most of the favorable classes such as mass depletion, gentle slopes and flatter ground with deep soils carry broad leave species such as *A. pindrow* and *P. wallichiana* attaining a good height and often form closed canopy here and there in the forest. The area is quite fascinating for ecological studies regarding its diversified topography which generate variety of habitats ranging from steep to moderate slopes and deeply incised valleys. Local vegetation differs at the two ends of the elevation scale as a result of variation in topography and soil moisture. The study of vegetation in these landscapes provided the basis to elucidate how vegetation composition changed with altitudinal gradient which generate climatic and edaphic variations which in turn affect the plant assemblage process at regional level. Undergrowth shrub almost present which are *C. montana, L. webbiana, P. jacquemontiana, R. webbiana, R. fruticosus, S. saligna* and *V. cotinifolium* as specific genera. Several evergreen shrubs such as *strobilenthus urticifolia plant* species of particular areas. No of genera which show Climber habitat such as *H. nepalensis, Rosa microphylla* and *J. humile* were frequent. This vegetation is same as present in other Northern part in America and Europe (Champion and Seth, 1968).

As a result of Cluster Analysis five associations are formed and plotted on axis 1 and axis 2 as represented in scatter plot. For data simplification different procedures should be used to give identical outcome. We take results to confirm the partially overlapping nature of associations define by ordination axis. The ordination axis may explain in some way the major ecological determinant which impacts the data of each stand and by using plants and environmental variables to explain the over-lapping characteristic. Basic ordination axis showing the position from high elevation to low elevation

from left to the right hand. The samples of the lower elevation are showed on the extreme right side of the diagram while the samples from high altitudes tend to find on the left side of the diagram. Not only is the elevation, but distribution of species also possibly affected by soil properties (Dasti *et al.*, 2007). DCA revealed that there is a good relation between soil form and association characterized by numeric reasoning along elevation gradient. The DCA outcome assume that elevation, pH, EC, organic contents, nitrogen, phosphorus, potassium, calcium, magnesium, sodium, chloride, salt and hydrogen carbonate are major factors for the diversifications and assemblage of floral species. This diversification of species along the elevation gradient influences the community organization (Gleason, 1917and 1926; Ramensky, 1924). Elevation is not amazing, but it is closely related with rainfall and distribution of rainwater. At higher altitude the run-off will produce then move downward and make changes in moisture contents which results changes in vegetation pattern. Therefore, movement of water in downslopes plays a main role in determining the zonation on peak point. Hence the initial ordination axis showed that change in moisture contents from top stony region (with higher score A, D, E), lower region (with lower score, B, C). Flora of the top zone consisted of species *C. nutans, C. nubigena, E. wallichii, G. boreale, H. maximum, J. humile, P. major, P. lanceolata, P. nepalensis, P. nepalensis, P. vulgaris, R. nepalensis, R. webbiana, T. repens, T. pratense, T. emodi, V. grandiflorum, whereas A. millefolium, Allaria petiolata, A. wallichianum, C. japonica, C. affinis, D. ramosa, E. angustifolium, F. indica, G. wallichianum, I. bicolor, L.cashmeriana, M. biflora, M. alpestris, N. govaniana, R. sceleratus, R. fruticosus, Scrophularia frutescence, S. chrysanthemoides, S. tomentosa, S. urticifolia, T. serphyllum, V. canescens* and *W. amherstiana* are confined to the lower altitude (Danin *et al*. 1975; Evenari *et al*., 1982; Dasti *et al*, 2010). So, it suggested that most of the variation in the plant assemblage structure is affected by altitude and soil chemistry (Wazir *et al.*, 2008; Saima *et al.,* 2018; Altaf *et al.,* 2020; Bibi *et al.,* 2020). Highest values of species richness and diversity were recorded after the monsoon rainfall because these are the months when plant species show the peak growth, and these results are consistent with the finding of Shaheen *et al.* (2011). Plant growth in these moist temperate forests starts with commencement of summer and melting of snow caps on the mountains. The growth activity depends on temperature regime and increases with increase in temperature and moisture during the month of August. Recently, Stevens, (1992) described that a gradual monotonic decline in species richness and diversity with increasing elevation is reflected a general pattern. In the present study, the SR follows this imperative significantly. However, it showed a significant negative correlation (r= 0.53) with the altitude. Rahbek (1995) conducted a research work on species richness patterns in relation to elevation and concluded that most of the studies perceived a mild-elevation peak in species richness. Similar findings were also reported by Grytnes and Vetaas (2002) in Nepalese Himalaya. Similar results have been reported by Shaheen *et al.* (2011).

Variation in elevation plays an important role in shaping the plant communities. Altitude represents intricate combination of related climatic variables closely correlated with several other environmental factors consisting of soil chemical properties, available nutrients contents and substrate constancy (Wazir *et al.,* 2008; Ramsay and Oxley 1997). Within one elevation the community structure is influenced by many other geo-climatic factors like temperature regime, topography variations and soil type. A significant negative correlation was detected between diversity and altitude. Similar results were reported by Shaheen *et al.* (2011). The staggered elevation distribution of the divisor or dominant species showed that dominants are similar to each other's major competent and essential determiner of each other's diversification (Whittaker, 1972). The tendency of certain plant families such as Lamiaceae, Asteraceae and Rosaceae are the specie rich about the entire elevation gradient (Gentry, 1988). The elevation and pH explain an important role in assemblage constitution. The species of association A present in soil with comparatively maximum pH. Although the results showed that pH decreases as altitude increase is associated with the plant size. It is unlikely that pH indirectly affects

the assemblage but there is evidence that pH decreases with the increase of altitude is due to downward motility of soil nutrients that impact the special organization along the elevation transect (Pendry and Proctor, 1996). The inter-actin of pH with distribution and social activity of flora is not a new thing but already has been explained by many workers. The correlation between species distribution with sodium, chlorine and potassium recommended that flora is a convoluted assemblage of substrates, experts and generalists. It is revealed that assortment and ordinance both are capable to delineate the plant associations with reference to their habitat. Topographic variations at the general level a major factor that determines the community structure in the mountain environment. The change in edaphic conditions associated with shape and give a good chance to conduct advance research, in the laboratory and as well as in the field.

5. Conclusion

The vegetation is complex collection of plant species. The interactions of plants of various species with each other and with their environment confirm that attempts to unravel species – environment relationship are found to be difficult. Rarely will there be clear – cut relationships, more usually the picture will be indistinct. The methods of vegetation analysis can only be regarded as providing possible initial pointers to important species- environment relationships. The results produced by analytical methods are only as good as the data collected in the field; sophisticated analysis cannot refine poor data, so therefore the importance of good field work included the devising of the sampling scheme, good species identification and accuracy in any subsequent laboratory work. Even when the most efficient of modern analytical techniques are applied to good data, the results contain a mixture of real trends together with fake ones generated by the underlying mathematical models which is never completely appropriate to the data in hand. The difficulty is in importance of undertaking further analytical work on the same data. In the present study, we took a rather simplistic approach by looking at only one environmental factor at a time. Where an ordination indicated the importance of several environmental factors at a time by their highly correlation on an axis, better results might have been obtained in the fellow-up if all the indicated environmental factors of importance had been examined together in some way. The present study highlights species richness, vegetation patterns and community composition in the open gap forest area at various altitudes. In the present investigation the significant correlation between altitude and DCA axis I suggested that ordination axis I appeared to be marked influenced from topography and thereof the redistribution of rainwater or melting snow which effect the plant assemblage and overall species richness. Significant negative correlation of species richness with altitude suggests the influence of these environmental factors on species richness and overall vegetation. The significant negative correlation of soil pH with DCA axis I suggest that sampling sites located at high elevation tend to accumulate less amount of bivalent ions (Ca and Mg) resulting in less release of H+ and a decrease in pH. Thus, the distribution of species along DCA axis I is determined largely by soil chemistry. However, further research is needed to elucidate the relationships. Beside of these difficulties mentioned above it may be concluded that both classification and ordination are able to delimit the plant associations according to their environment. However, many ideas can be generated by such a result, but they are only hypothetical. Much more work, both in field as well as in laboratory is needed to reveal the clear picture.

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Community Composition and Species Diversity in an Upland Conifer Forest: A Case Study in Rajwal Forest, Kaghan Valley, Pakistan

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Abstract

The present research work was conducted in Rajwal Reserve Forest, which is the part of moist temperate forests of western Himalaya, Pakistan with a rainfall from 400 mm to 800 mm. The altitudinal range is from 1500 m to 3000 m (a. s. l). The floristic variation in these Himalayan forests is poorly understood especially in the study area. Vegetation from 30 stands in between 1500 –3000 m altitude was sampled. A total of 79 species, belonging to 24 genera and 13 families from 30 stands were recorded. Soil samples were collected to document edaphic conditions. Soils were chemically analysed. Ordination (DECORANA) and classificatory techniques were used to analyse the vegetation data. Soil chemical properties were significantly (P= 0.05; F=2.11) associated with the vegetation variation along an altitudinal gradient.

Keywords: Floristic Composition; AltitudinaL gradient; Multivariate analysis

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1. Introduction:

Rajwal Forest is the part of moist temperate forest formation in W. Himalaya, Pakistan and situated between latitudes 34o 54' 26 N, to 35o 08' 76 N and 73o 38' 90 E to 74o 01' 30 E longitude. This forest is comprised of diverse macro- and microclimatic conditions that harbor a great diversity of life forms. The vegetation in most part the forest is greatly affected by different factors, which play important role in the distribution and composition of plant communities. These factors included variations in topography, run-off generation and pattern of distribution, altitudinal variation in temperature and soil properties. Climatically the area is classified as temperate continental with an average frost-free growing period of 204 days extending from April to October. Environmental gradient along with their complex interaction affect the vegetation structure and composition (Tadesse *et al.,* 2008; Saima *et al.,* 2018) and hence, studying environmental gradient analysis is important to understand factors contributing the important role in composition, distribution pattern and dominance of plant species. Especially elevational gradient influences the floristic composition and changes community composition accompanied with variation in climatic regime (Beg *et al.,* 1996). Beside the importance of elevation, soil topography, seasonal variations in temperature and precipitation profile determines the distribution of plant communities. In western Himalaya, Species composition, community structure, and function are the most important ecological aspects of forest ecosystems, which fascinate the ecologist for different studies of these characteristics (Khan *et al.,* 2010; Shaheen *et al.,* 2012). Most of Botanist, ecologist studied the phytogeography of Himalayan Mountains and described the valuable information (Tadesse *et al.,* 2008). The detailed floristic and bio-geographic information about areas of Western Himalaya is poor.

A number of workers have conducted vegetation surveys in different parts of northern areas of Pakistan. Champion *et al.,* (1965) and Beg, (1975) explored the vegetation of the area and classified various types of forests and on the basis of elevation and temperature classified forest types into different vegetational zones. Ahmed, (1976) done similar studies, who conducted multivariate vegetation analysis of Skardu. Ahmed, (1986) described vegetation of foothills of Himalaya range in Pakistan. Hussain and Illahi, (1991) studied ecology and vegetation types of Lesser Himalayan of Pakistan. Ahmed *et al.,* (2006) presented phytosociology and structure of Himalayan forests from different climatic zones of Pakistan. Shujaul *et al.* (2011) described the species and community diversity of vascular plants of Naran valley. Such types of studies have been conducted by Ahmad *et al.* (2010), (2011), Shaheen *et al.* (2011), Saima *et al.* (2009), (2018) and Hashim and Dasti, (2019). Nazir *et al.* (2021) described the vegetation composition of Kamal ben, Khanyan Reserved Deodar Forest.

In the present research work efforts have been made to analyze soil characteristics in detailed and vegetation patterns across the elevation gradient. The study attempts to provide altitudinal changes in the vegetation of Rajwal temperate forest, Kaghan valley. The ecological feature of this valley includes position of particular geography, promoted plant diversity and presence of meadows in alpine and sub alpine range. The mountainous tracts have very difficult topography and inaccessibility. So, studies of vegetation present on it are very difficult. That is why the survey of flora and vegetation structure was incomplete. Classifying the natural ecosystem into its associations and habitat types of natural recourses is important for long term forest management measures. Current knowledge of the relationship between vegetation type and environment is mostly descriptive (Khan *et al.,* 2013). Comprehensive detailed species composition and its response to their ambient environment is poorly known. However, an integrated observation of composition and structure of temperate forests in Pakistan is still deficient; the present study is a part to finalizing the overall vegetation picture of these areas.

1.1. Objectives:

- To elucidate the correlation between species distribution and edaphic factors.
- To determine the distribution of species richness and diversity along the altitudinal gradient.
- To determine the plant associations and the limit of their boundaries.

2. Materials and Methods:

2.1. Study Area:

The study area is located between 1805 m to 2200 m elevation (a. s.l.) in Kaghan valley, District Mansehra. It is a part of Western Himalayan range in Pakistan located between 34° 54.49 N´, latitude and 73°37. 88 E´ longitudes. In the valley Kunhar River, which flows from Lulusar Lake enters to the river Neelam. The valley is deep about 96 km long and hardly more than 24km wide and is bounded with lofty mountains that remained snow covered most part of the year. The mountain massive harbor several glaciated lakes.

2.2. Climate:

The study area is cold and remains covered with ice most of time of the year. The temperature in the winter decreased to -9 Co. In the summer, the heat is intense during the day and at night temperature drops to zero. The mean annual rainfalls vary from year to year and mean precipitation ranges between 860 mm to 1290mm. In early autumn, chilly winds bring the temperature down. Precipitation is occurring in spring summer and snow in autumn, winter.

2.3. Vegetation and Soil sampling

In wet temperate forests extensive vegetation sampling and identification of species were very laborious and time consuming. As in canopied forests number of species involved was very high, so it was difficult to obtain full ecological parameters. To be able to cover larger spatial extent in more detail, only two plant groups: Pteridophytes (Ferns) gymnosperms and Dicotyledonous (Angiosperms) under-story species were explored in detail. Grasses belong to Poaceae were ignored because of their insignificance occurrence in the study area. The study area was divided into 30 sites (stands), to explore the entirely and to cover the complete floristic variation in area. All the stands were selected systematically along the accessible altitudinal gradient through the forest. These stands were studied during monsoon season. A single transect was used instead of discrete plots because continuous sampling allows observations of spatial change to be made and compared between data sets, and assessment of whether turnover is spatially continuous or occur more rapidly at certain points.

The altitudinal profile transect was measured using an altimeter, Measurements were taken for every sampling unit (stand). Surface soil samples (the top 5 cm of the mineral soil) were collected at each site (n=20). Each of the soil samples consisted of five pooled sub-samples collected from each stand. Soil analysis was carried out using standard procedures (Richards, 1954; Hussain, 1989).

2.4. Diversity

Species richness diversity indices were assessed, and the Shannon index of diversity (H) and the Evenness index (j) calculated in all sampled reserves, for each species. Taxa richness (S) was defined as the total taxa number present in a reserve or site (Magurran, 1988).

2.5. Data Analysis:

2.5.1. Vegetation Analysis

The presence/ absence data of the plant species obtained during field were converted into frequency before performing numerical analyses. The relative values of the frequency of divisive species were used for analysis. The number of species in each community was considered as species richness.

2.5.2. Classification and Ordination

To assess the variation in the distribution pattern of plant communities, a gradient analysis was performed using the MVSP program. To classify the sampling units based on their floristic similarity, the cluster analysis was performed. Due to the similarity between the communities, the Spearman correlation coefficient was used. The multivariate statistical package (Kovach, 1999) was used both for grouping and ordering.

2.5.3. Soil Sampling and Analysis

Soil samples (approximately one kg each sample) collected from each site were packed in a polythene bag and labeled by tagging sticker. The samples were air-dried and passed through a 2mm sieve to remove the debris of roots and seeds. The portion of soil finer than 2mm was used to analyze for determination of available cations and anions: Calcium, Magnesium, Nitrogen, phosphorus and organic matter, pH, EC and organic carbon using standard methods (Richards, 1954). The variations in soil properties among plant communities were determined by analysis of variance (ANOVA). Appropriate graphs were designed to explain the differences between the plant associations recognized by cluster analysis. The correlation of soil parameters with scores on axis 1 and on axis 2 of the arrangement was calculated according to Pearson correlations. The calculations were carried out with the help of MINITAB, a statistical software package.

3. Results:

3.1. Species Richness and Diversity

Species diversity was calculated by Shannon's diversity indices. Possible values of diversity ranges between 1.0 to 1.3 and do not exceed 2 while maximum values designate high diversity (Table No. 1). Analysis of variance showed significant difference in Shannon's Diversity indices along altitudinal transect (Table No. 5), although the mean values differ significantly between the communities, the total number of species tend to be decrease with increase in altitude. Maximum number of species was recorded (24 species) in community type located at lower elevation (1800 m a.s.l) and minimum values for species richness (19) were recorded in community type located at high

elevation (2178 m a.s.l.). While the community type found mid of the elevation showed in between values for species richness (23).

Table 1. Species diversity Indices in three community type recognized by normal cluster analysis.

Variables		Associations			*F & P – value*
		A	B	C	
Sp. Richness	Mean	24	23	19	2.63
	Std.	4.79	5.47	2.58	P<0.05
Sp. Index	Mean	1.30	1.12	1.00	2.11
	Std.	0.05	0.04	0.11	P<0.05

3.2. Classification

The results of the cluster analysis are presented in (Figure 1). The first hierarchical level separated the dry temperate mid slope community (A) from the wet temperate communities (B-C). The second hierarchical level delineated the wet temperate top slope community (B) from the rest. As a result of three levels three clusters (groups) were created. These clusters identified three types of vegetation.

3.2.1. Association A Group 1

Group 1 is marked by rich ground cover of herbaceous perennial *Alliaria petiolata, Equisetum arvense, Impatiens bicolor, Oxalis corniculata, Ranunculus laetus, Convolvulus arvensis*, which were altogether absent from the other two associations (Table 2) and located at low slopes. *Aconogonon alpinum, Capsella bursa pastoris, Cotoneaster affine, Dactylis glomerata, Galium aparine, Geranium rotundifolium, Impatiens edgeworthii, Impatiens sulcata, Indigofera atropurpurea, Pimpinella diversifolia, Leontopodium alpinium, Myosotis alpestris* were frequent species in this association delineated by normal cluster analysis, *Oxyria digyna, Myricaria squamosal, Malva neglecta, Lolium rigidum, Juncus thomsonii, Trifolium rapens, Urtica dioica, Trifolium rapens, Nepeta erecta, Cynoglossum glochidiatum* and *Prunella vulgaris* were rare species in this association (Table 2).

3.2.2. Association B Group 2

Group 2 is distinctive in having *Lactuca brunoniana, Lavatera kashmiriana, Lepidium latifolium, Paeonia emodi, Salix acmophylla,* which were altogether absent from the rest recognized by Normal cluster analysis (Table 2). *Adiantum venustum, Erigeron multicaule, Fragaria nubicola, Geranium wallichianum, Plantago lanceolate* and *Rosa webbiana* were dominant species in this association. *Juncus thomsonii, Lolium rigidum, Malva neglecta, Viburnum cotinifolium, Trifolium rapens, Stellaria aquatic, Sambucus wightiana, Planta golanceolate, Nepeta erecta, Cynoglossum glochidiatum, Chenopodium foliosum, Dioscorea deltoidea, Polygonum paronychioides, Polygnum aviculare, Poa alpine, Pimpinella diversifolia,* were found frequent species recognized by the normal cluster analysis. *Paeonia emodi, Lavatera kashmiriana, Lactuca brunoniana, Myosotis alpestris, Prunella vulgaris, Ranunculus arvensis, Silene indica and Oxytropis microphylla* were rare species (Table 2). The shrub layer in this group is characterized by I*ndigofera atropurpurea,* and *Rosa webbiana* (Table 2).

Table 2. Frequency percentage species in groups (1-3) identified by Normal cluster analysis.

Species Names	Association A	Association B	Association C
Alliaria petiolata	1.41	--	--
Equisetum arvense	0.28	--	--
Impatiens bicolor	0.85	--	--
Oxalis corniculata	1.98	--	--
Ranunculus laetus	0.85	--	--
Convolvulus arvensis	1.13	--	--
Primula rosea	3.11	--	--
Lactuca brunoniana Wall.	--	0.38	--
Lavatera kashmiriana Lamb.	--	0.38	--
Lepidium latifolium L.	--	0.76	--
Paeonia emodiWall.ex.HK.	--	0.38	--
Salix acmophylla Boiss.	--	3.44	--
Bistorta amplexicaulis D. Don.	--	1.15	2.08
Bromus japonicas Thunb.	--	1.91	3.13
Caltha palustris L.	--	0.38	2.08
Elsholtzia spp	--	1.53	3.13
Festuca L.	--	1.15	1.04
Aconogonon alpinum All.	0.56	0.76	--
Capsella bursa pastoris L.	0.56	0.76	--
Cotoneaster affine Lindly.	1.41	2.29	--
Dactylis glomerata L.	0.28	0.76	--
Galium aparine L.	1.69	0.76	--
Geranium rotundifolium L.	1.69	3.44	--
Impatiens edgeworthii Hook.	0.28	0.76	--
Impatiens sulcata Wall.	0.56	1.15	--
Indigofera atropurpurea Ham.	2.82	3.82	--
Pimpinella diversifolia DC.	1.69	1.53	--
Leontopodium alpinium Cass.	1.13	0.38	--
Myosotis alpestris F.W.	1.98	0.38	--
Poa alpina L.	2.82	1.15	--
Podophyllum hexandrum Royle.	0.56	0.76	--
Polygnum aviculare L.	2.82	1.91	--
Polygonum amplexicule D.Don.	1.41	0.76	--

Species Names	Association A	Association B	Association C
Polygonum paronychioides C.	1.13	1.15	--
Prunella vulgaris L.	0.28	0.38	--
Ranunculus arvensis L.	0.85	0.38	--
Cyperus L.	0.56	1.15	--
Dioscorea deltoidea Wall.	3.11	1.15	--
Silene indica Roxb.	3.11	0.38	--
Epilobium cylindricum D.Don.	0.28	--	1.04
Plantago major L.	0.28	--	1.04
Dianthus jacquemontii Edgew.	1.13	--	3.13
Pedicularis L.	1.41	--	4.17
Adiantum venustum D.Don.	1.13	2.29	4.17
Chenopodium foliosum Asch.	0.56	1.15	1.04
Cirsium falconeri Hook.f.	1.69	0.76	3.13
Conyza japonica Less.	1.41	0.76	1.04
Cynoglossum glochidiatum Wall.	0.85	1.91	3.13
Dryopteris ramosa C. Hope.	3.11	2.67	2.08
Erigeron multicaule Wall.	1.41	3.05	1.04
Fragaria nubicola Hook.	3.39	3.05	5.21
Geranium wallichianum D. Don.	3.39	3.05	2.08
Micromeria biflora Buch.	2.82	2.29	5.21
Nepeta erecta Bth.	0.56	1.15	3.13
Phlomis bracteosa Royle.	2.54	3.44	1.04
Plantago lanceolata L.	2.26	1.91	2.08
Rosa webbiana Wall.	2.26	3.05	2.08
Rumex nepalensis Spreng.	1.98	2.29	1.04
Sambucus wightiana Wall.	1.69	1.15	2.08
Stellaria aquatica Scop.	1.13	1.53	1.04
Taraxacum officinale Webb.	3.39	2.67	4.17
Trifolium rapens L.	0.28	1.53	2.08
Urtica dioica L.	0.28	0.38	1.04
Veronica bilobia L.	0.28	0.76	2.08
Veronica serpyllifolia L.	3.67	3.05	4.17
Viburnum cotinifolium D.Don.	3.67	1.53	2.08
Viola biflora Wall.	--	2.29	1.04

Species Names	Association A	Association B	Association C
Filipendula vestita Wallich.	1.13	0.76	2.08
Heracleum cachemiricum C.	1.41	0.38	1.04
Juncus thomsonii Buchen.	0.56	1.91	2.08
Lolium rigidum Gaud.	1.41	1.15	1.04
Malva neglecta Wallr.	0.85	1.91	1.04
Myricaria squamosal Desv.	0.56	1.53	3.13
Oxyria digyna L.	0.85	1.53	1.04
Oxytropis microphylla Palla.	2.26	0.38	1.04
Phragmites karka Retz.	--	1.15	4.17
Sporobolus arabicus Boiss.	2.26	0.38	1.04
Thymus linearis Benth.	0.85	1.53	2.08
Tragopogon pratensis L.	--	2.29	2.08

3.3. Gradient Analysis of understory vegetation:

The gradient analysis was used to identify a minimal set of variables that elucidates the species distribution patterns (TerBraak, 1992). The DCA was applied in analysing the geo-climatic variables, effect the structure and composition of herbaceous flora. The DCA was performed on full set of 30 sampling sites. The chosen environmental variables of altitude, soil chemistry, rainfall, and average temperature significantly affect the flora. The two axes obtained by ordination explained the variation in the flora of the study area. DCA axes scores confirming the importance of the variables. The groups of plant communities resulting from the DCA using the floral presence / absence corresponded with their geographical or topographical position. Each plant community type had a distinct floristic composition of the under-story vegetation. Some weedy species were frequent in the whole of the study area but restricted in distribution. The eigen values of the Axes are given in (Table 3).

Table 3. Eigen values and Cumulative percentage values of DCA axis 1&2 obtained by ordination.

Axis	Eigen value	Percentage of total	Cumulative percentage
1	0.275	11.736	11.736
2	0.155	7.483	19.219
3	0.232	6.088	26.559
4	0.191	5.007	31.566

The Eigen values of Axes 1 & 2 (Figure 1) were considerably high as compared to lower order Axes, and that later were flouted. The scores of axes1shows significant correlation with soil properties, elevation and other parameters investigated in the current research work. The result proposed the overriding importance of elevation as environmental factors encounter the shaping of plant assemblage and community composition along the elevation gradient in a forest mountainous ecosystem. Beside the elevation gradient, the first DCA Axes was significantly correlated with most of the soil variables.

Suggesting the effect of substrate which modules the vegetation structure and composition. On the other hand, axes 2 is multifaceted and significantly negatively correlated to Electrical conductivity (P=0.01). However, most of the edaphic factors were positively correlated with DCA axes 1. The diversity indices were not correlated significantly to the DCA axes 2 (Table 4).

Table 4. Correlation between DCA 1 & 2 axis scores, soil variables, and elevation and diversity indices in Rajwal reserved forest.

Factors	Axis 1		Axis 2	
	Coefficient	Significance	Coefficient	Significance
Climatic Variables				
Altitude (m.a.s.l)	0.890	0.000***	-0.07	0.899
Soil Variables				
E.C (dS/m)	-0.15	0.528	-0.53	0.01*
pH	0.09	0.685	-0.11	0.647
Sodium (Na) (meq/L)	-0.45	0.049*	0.29	0.206
Nitrogen (%)	0.57	0.009**	0.18	0.453
Chloride (meq/L)	-0.52	0.018*	-0.12	0.614
Phosphorus (ppm)	0.176	0.458	0.04	0.854
Ca+ (meq/L)	-0.49	0.026*	-0.19	0.421
Organic Matter (%)	0.505	0.023*	0.16	0.482
Diversity Indices				
Species Diversity	-0.003	0.989	-0.13	0.587
Species Richness	-0.03	0.901	-0.07	0.747

The results of DACORANA depicted in (Figure 1) put forward that the species like *Bistorta amplexicaulis, Bromus japonicas, Caltha palustris, Elsholtzia and Festuca* species situated at the right side of the diagram (maximum values of axis scores represent high altitude and poor soil nutrients and electrolytes). Whereas the species like *Alliaria petiolata, Equisetum arvense, Impatiens bicolor, Oxalis corniculata, Ranunculus laetus, Convolvulus arvensis* placed at the left side of the DCA axes (Low score on ordination plot are normally found at low altitude (Table 5). The majority of the species found in the mid of the elevation *Lactuca brunoniana, Lavatera kashmiriana, Lepidium latifolium, Paeonia emodi, Salix acmophylla* are found in soils showing moderate concentration of soil anions and cations. This occurrence of the species across the elevation gradient suggested that soil chemical properties played an important role in the spatial distribution of species.

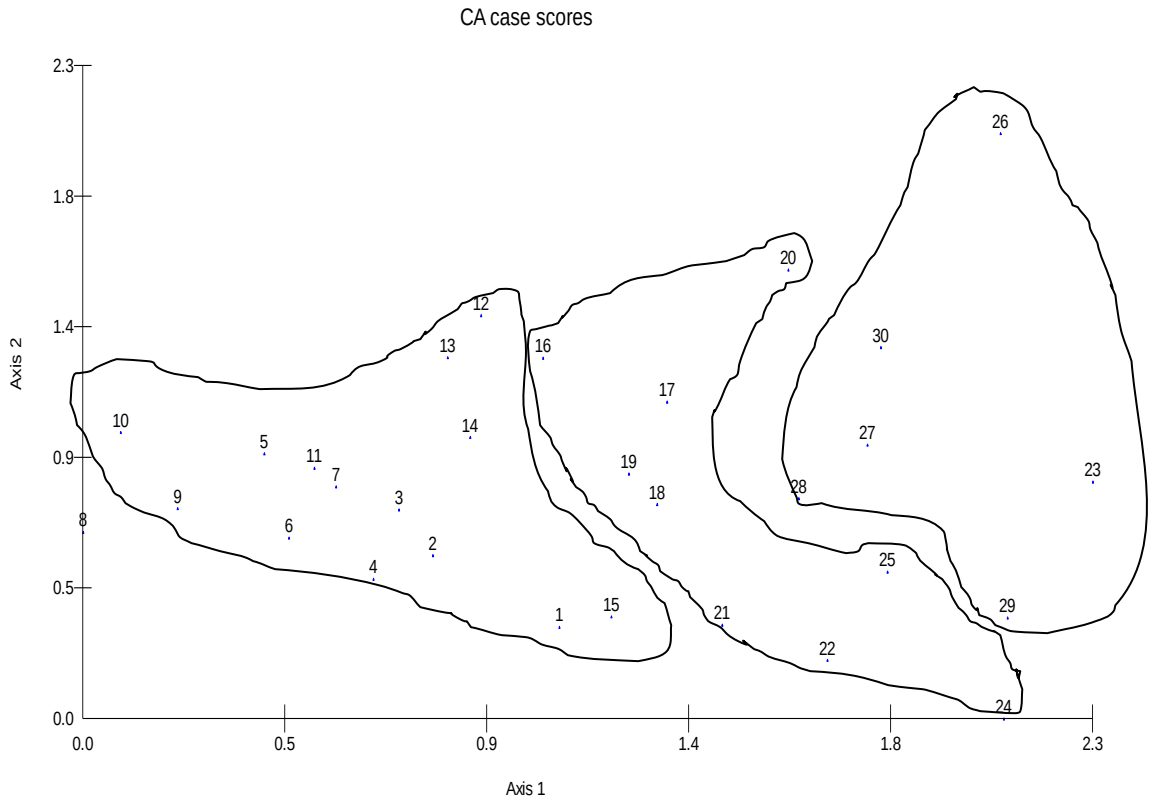

Figure 1. The DECORANA (Axes 1 & 2) plot of 30 samples from the study area (Rajwal Reserved Forest).

Table 5. Mean values and standard deviations (SD) for all soil variables for the three associations identified by the Normal Cluster Analysis.

Factors	Statistical factors	ASSOCIATIONS			F-value
		A	B	C	
Altitude (m.a.s.l)	Mean	1838.83	1935.4	2146.4	73.81
	Std.	54.10	43.9	20.1	P<0.000
E.C (dS/m)	Mean	2.04	1.16	0.99	28.31
	Std.	0.11	0.30	0.46	P <0.000
pH	Mean	7.47	6.97	6.70	16.62
	Std.	0.33	0.10	0.09	P <0.000
Sodium (meq/L)	Mean	12.97	10.23	8.10	33.96
	Std.	1.18	3.18	3.38	P <0.000
Nitrogen (ppm)	Mean	0.13	0.12	0.11	1.39
	Std.	0.00	0.01	0.00	NS

Chloride (meq/L)	Mean	51.60	50.63	49.28	14.95
	Std.	0.00	0.04	0.12	P <0.000
Ca/Mg (meq/L)	Mean	16.74	18.79	24.11	20.76
	Std.	4.52	3.36	11.24	P <0.000
Organic matter (%)	Mean	2.10	2.04	1.90	1.95
	Std.	0.08	0.03	0.34	NS
Phosphorus (ppm)	Mean	19.40	18.80	16.40	0.60
	Std.	2.28	4.72	4.72	NS
Potassium (ppm)	Mean	2.93	1.92	1.22	6.51
	Std.	0.26	0.53	0.05	P <0.000

4. Discussion

The Rajwal reserve forest was covered by coniferous gymnosperms and broad leaves trees: *Acer caesium, Populus cilata,* and *Quercus dilata*. The tree strata consist of *Pinus wallichiana, Fir, Spruce* and *Deodar species* (Champion and Seth, 1965). *Rosa webbiana Vibernum cotinifolium, Berberis kunawrensis, Cotneaster affine and Spirea vaccinifolia* contribute to shrub. The ground strata is dominated by *Micromeria biflora, Fragaria nubicola, Traxacum officinale, Phragmittes species, Poa alpine, Nepeta erecta, Polygnum aviculare, Impatiens edeworthii, Fragaria indica, Galium boreal, Geranium rotundifolium* and *Rumex nepalensis* were the frequent herbs similar findings were recorded in most temperate forest of western Himalaya and similar finding were recorded by Ahmad *et al.* (2006), Noor *et al.* (2013), Shaheen *et al.* (2016), Ahmad *et al.* (2016) and Saima *et al.* (2018).

The three communities were recognized by using the Clustering analysis. Similar results were obtained by Gradient analysis using DCORANA. The two protocols of data interpretations can have similar results. The first ordination axis exhibited a gradient from low elevation to high elevation from left to the right hand in the diagram. The plant which survives at high elevation localities are distributed on the extreme right side of the ordination diagram while the plant species which enjoy low elevation forests tend to express on the left side of the ordination diagram. Besides the elevation the distribution pattern of the species is shaped by soil physical and chemical characteristics (Ahmad *et al.,* 2006; Saima *et al.,* 2009; Khan *et al.,* 2011; Saima *et al.,* 2018). Soil chemical reactions (pH) mostly decreased with elevation and the most acidic soils are generally found at high altitude (Table 5). Similar results were conducted by Pendry and Proctor, (1996) but opposite to the results of Veneklaas, (1991) who stated maximum values of soil pH in sampling stand distributed along lower elevations. Soil organic matter showed also decreasing behavior with elevation (Table 5). Soil cations and anions showed the decreasing trends along with elevation gradient similar results were expressed by Pendry and Proctor, (1996), Tanner *et al.* (1998) and Saima *et al.,* (2018).

The Detrended correspondence analysis results showed that elevation variation, soil chemical reactions (pH), soil electrical conductivity, soil cations and anions play significant role for shaping the composition of plant communities. The importance of altitude is not astonishing but is significantly encountered with precipitation in the form of rainfall. Vegetation of Association A belong to *Alliaria petiolata, Equisetum arvensis, Impatiens bicolor, Oxalis corniculata, Ranunculus laetus, Convolvulus arvensis* and *Primula rosea.* These species were absent at higher altitudes. On the other hand *Lactuca brunoniana, Lavetra Kashmiriana, Lepidium latifolium, Paeonia emodi, Salix acmophylla,* confined to the Association B located at mid altitude, Similarly *Bistorta amplexicaulis, Bromus japonicas, Caltha palustris and Festuca* species were only distributed in Association C located at high altitude and were absent

from the rest approving the importance of elevation in shaping the plant associations (Pendry and Proctor, 1996; Khan *et al.,* 2013; Shaheen *et al.,* 2016; Saima *et al.,* 2018). The consistent negative correlation between altitudes, edaphic and geo-climatic factors suggested that some combine effect is important, and the species richness and diversity distribution patterns tend to increase with rise in precipitation and drop with low temperature at high elevation. So, the plant communities which located at high elevation have high richness and diversity (Korner, 1998; Khan *et al.*, 2011), to increases with soil fertility in forests which are located at low elevation while decrease with declining concentration of anions and cations (Pendry and Procter, 1996), to exhibited decreasing behaviour with increase in winter snowfall (Sheikh *et al.*, 2002; Shaheen *et al.*, 2016) and to show decreasing trend with increase in soil acidity (Pendry and Procter, 1996).

The correlation statistical analysis of species distribution with soil soluble cations and anions proposed suggested that vegetation showed a complex behaviour in term of distribution, some species showed site specification preferences, some are specialist, and some are generalists in harbouring ecological landscape. It can be suggested that both classification and ordination analysis determine the plant communities according to their Habitat. Topographic variations are important factor that maintains the community structure in the mountain habitat and provide an opportunity for further research work, both in the field and laboratory.

5. Conclusion

The current research work reveals the species richness, diversity patterns and community establishment in the Rajwal reserve forest area along elevation gradient. Our results described that species richness and diversity exhibited decline behaviour at higher elevations. In the present study the significant correlation among altitude and DCA axis I suggested that ordination axis I appeared to be marked influenced from topography and thereof the redistribution of rainwater or melting snow which effect the plant assemblage and overall species richness. Significant negative correlation of species richness with pH and positive with altitude suggests the influence of these environmental factors on species richness and overall vegetation. The significant negative correlation of soil pH with DCA axis I suggest that sampling sites located at high elevation tend to accumulate less amount of bivalent ions (Ca and Mg) resulting in less release of H+ and a decrease in pH. Soil EC of the sampling plot showed a significant but negative correlation with DCA axis II. Consequently, the distribution of species along DCA axis II is determined largely by soil chemistry. Beside of these difficulties mentioned above it may be concluded that both classification and ordination are able to delimit the plant associations according to their environment. However, many ideas can be generated by such a result, but they are only hypothetical. Much more work, both in field as well as in laboratory is needed to reveal the clear picture.

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Bioassay Test of Allelopathic Potential of Sunflower (*Helianthus annuus* L.) against Mung bean (*Vigna radiata* (L.) R. Wilczek)

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Abstract

The Sunflower (*Helianthus annuus* L.) has been well- documented for its high allelopathic potential against different weeds and crop plants. The allelochemicals released by *Helianthus annuus* influenced the germination and growth of other species. Laboratory and greenhouse experiments were conducted to elucidate the allelopathic potential of *H. annuus* against *Vigna radiata.* In this research study laboratory experiments comprised of petri- plate trials which were accomplished by the application sunflower whole plant extracts in different concentrations as 4%, 8% and 12% extract to the germinating seedlings of test crops while the control seedlings were treated with distilled water. Greenhouse experiments were distinguished by whole plant powder was incorporated with soil in different amounts like 4, 8 and 12 g applied to the potted plants, whereas control plants were only filled with soil. The growth parameters like percent germination, germination index, radicle and plumule elongation, seedlings growth, fresh and dry weights and chlorophyll contents of test crop were significantly inhibited by the application of extracts as well as with powder 12 % extract showed greatest inhibition in the growth parameters of test crop. During this research study, the plumule lengths of *V. radiata* were much more affected by the extract concentration as compared to control. Powder extract also contained phytotoxic compounds that caused remarkable reduction in plant heights and chlorophyll contents of test species. It was obvious during this research study that *V. radiata* was resistant against the phytotoxic effects of sunflower. So, it can be concluded from this research observed the allelopathic effects of sunflower extract and powder showed inhibitory effects on different growth parameters which all are essential for plant growth. The main objective of present study is to

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explore the allelopathic effects of sunflower against mung bean, to determine the bioassay test of different plant parameters of test crop and allelopathic effect during plant growth.

Keywords: Allelopathy; Sunflower; Mung bean; Germination

1. Introduction:

The sunflower (*Helianthus annuus* L.) is member of family Asteraceae. It is the most important oil seed crop having high nutritional values. The allelopathic activity of Sunflower has been reported on subsequent crops as well as on weeds (Macais *et al*, 2002). Sunflower cultivation is done throughout the world as well as in Pakistan as it is a main source of vegetable oil used for different purposes. In Pakistan, it is cultivated during March and April and harvested in June. Sunflowers can be very large and require plenty of space although there are some varieties which have been specially bred to be compact for growing in smaller spaces or for growing in containers (Ali, 1977).

The sunflower has been reported highly allelopathic plant. Most of the members of Asteraceae family are well recognized as allelopathic crop plants on other plants to reduce the germination and seedling emergence of subsequent crops (Bialy *et* al., 1990; Muehlchen *et al.,* 1990). The allelopathic activity of Sunflower was first time accounted by Leather (1983) and Anjum and Bajwa, (2007). However, Macais *et al*. (2002) and Vyvyan (2002) reported that various phenolic compounds and terpenoids are found in sunflower cultivars. Several phytotoxic allelochemicals that have been identified in sunflower residues are chlorogenic acid, isochlorogenic acid, scopolin and alpha-napthol derivatives described by Wilson and Rice, (1968). Many other researchers Carter, (1978), Anderson *et al.* (1978), Gawronska *et al*. (2002), Bogatek *et al*. (2006), and Kupidlowska *et al.* (2006) also reported the highly allelopathic potential of Sunflower on weeds as well as several crop plants.

The test crop Mung beans (*Vigna radiata* (L.) R. Wilczek) is one of the most important, short-season, summer growing leguminous crop cultivated widely throughout the tropics and subtropics (Thomas *et al.,* 2004). It is native to Asia but widely cultivated in Africa and Latin America (Tomooka *et al.,* 1992; Saravanakumar *et al.,* 2004). It is one of the most important pulse crops of Pakistan that fulfils the nutrition requirement of almost every region. It is mainly cultivated in Southern Punjab and Sindh province during spring season (Ali, 1977). The *V. radiata* is ranked as the second most drought resistant crop after soybean in Asia. It has high nutritional values and has higher protein contents and better digestibility than any other pulse crop (Tabasum *et al.,* 2010). The grains of Mung bean contain 51% carbohydrates, 26% protein, 10% moisture and 3% vitamins. Asaduzzaman, (2008) reported that the remaining of Mung bean are also used as feed for animals as well as enhance the soil fertility. The allelopathic effects of *H. annuus* on several weeds as well as crop plants has been studied. Bashir *et al.* (2012) described that *H. annuus* have allelopathic potential on growth of Rice and Wheat.

1.1. Objectives:

- To explore the allelopathic effects of sunflower against mung bean.
- To determine the bioassay test of different plant parameters of test crop and allelopathic effect during plant growth.

2. Materials and Methods:

2.1. Experimental Design and Collection of Material

The experimental work was conducted in research laboratory and green house of Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan. The seeds of Sunflower and Mung beans were collected from a local Seed market the purity and germination of these seeds were 90 to 95 %. The field of Sunflower plants was cultivated in the field of Department of Botany, as the plants became young before flowering, the plants were collected.

2.2. Lab Experiment-Preparation of whole plant powder extract

The collected plants were air dried in green house, after drying plants were ground by Wiley mill (Thomas Wiley Lab Mill, Model 4). For the preparation of aqueous extracts, add 4, 8 and 12 g whole plant powder in marked beakers and 50 ml of distilled water was liquescing in each beaker for left the suspension for the time of 24 hours. After 24 hours, these suspensions were centrifuged by centrifuge machine and filtered through Whattman No.1 filter paper and add distilled water to make up the volume up to 100 ml of each concentration and kept the solution into marked conical flasks. All the glass wares were sterilized in autoclave at 121^{o}C and 15 lb pressure for 30 minutes to avoid microbial contamination.

2.3. Petri-Plate Experiment

The seeds of *V. radiata* were surface sterilized with 0.1 % mercuric chloride solution for 1-2 min and washed with distilled water. All the Petri plates were marked as control, 4, 8 and 12 %, along with the 5 replicates, respectively. Total 10 sterilized seeds of both crops were kept in petri-plates along with uniform distance. 2 ml plant powder extracts were poured in the replicates of each concentration while distilled water was poured as a control. The extracts were poured at alternate days. The petri plates of both crops were placed at room temperature in laboratory. The germination of seeds was recorded regularly on daily basis, while the radicle and plumule lengths were taken in alternate days.

2.4. Pot Experiment

Pot experiments were conducted in green house and in an open field of Department of Botany. The sandy loam soil and natural humus fertilizer was used in the ratio of 8:2. The soil was free from fungal population, insects, and other pathogenic organisms. All the pots were marked as control, 4, 8 and 12 g. Total 500 g soil was weighed and incorporated with plant extract powder, while the control pots were filled only with 500 g soil. Pots were kept in green house and irrigated for 2-3 days, and pots were prepared for sowing after 3 days.

2.5. Germination and Plant Growth

The seeds of *V. radiata* was surface sterilized as described previously. Total 10 sterilized seeds were sown in each pot. The germination record was noted on daily basis. After 8 days of germination, the plants were thinned and only four plants were left in each pot. After thinning, the plants height was measured weekly. All the pots were kept in open field due to required critical day light.

2.5.1. Germination Percentage

$$\text{Germination } (\%) = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2.5.2. Germination Index

The Germination Index was calculated by given formula of Khandakar and Bradbeer, (1983).

$$\text{Germination index } (S) = [\frac{N1}{1} + \frac{N2}{2} + \frac{N3}{3} \ldots\ldots\ldots] \times 100$$

Where S is the germination index, N1/1, N2/2.... are the ratio of number of the seeds germinated per day

2.5.3. Seedling Vigour Index:

The Seedling Vigour Index (S.V.I.) was calculated according to the following formula of Abdul-baki and Anderson (1973).

$$S.V.I = (\text{Root length} + \text{Shoot length}) \times \text{Germinsation } (\%)$$

2.5.4. Inhibition Percentage (%)

Inhibition percentage was calculated by following formula of Surendra and Pota, (1978).

$$\text{Inhibition } (\%) = 100 - (E2|E1) \times 100$$

Where, **I=** % Inhibition, **E1=** Response of Control plant, **E2=**Response of Treatment plant

2.6. Germination and Plant Growth

The pot experiment was terminated after one month and all plants of each pot of experiments were uprooted carefully for taken fresh weight, dry weight, detection of chlorophylls content and carotene.

2.7. Detection of Chlorophyll Contents

2.7.1. Preparation of Samples

For preparation of samples to detection of chlorophyll content, 2g fresh leaves of each concentration (control, 4%, 8% and 12%) were carried out. These leaves were crushed with 2 ml of 80% acetone in pestle and mortar, and then filtrate the extract with the help of muslin cloth. The extract and 2 ml of 80% acetone was centrifuged for 2-3 minutes at 1500 rpm. The supernatant was poured in a separate test tube and debris were washed with 2 ml of 80% acetone. The solution was centrifuged, and the supernatant was transferred into the same test tube. The procedure was repeated

until the debris become colorless. The pestle and mortar were rinsed with 80% acetone and collect the residuals of washing in the same test tube. Make up the volume up to 10 ml with 80% acetone.

2.7.2. Determination of Chlorophyll Content

The chlorophyll content (chlorophyll a, chlorophyll b and carotenoids) was determined by using Spectrophotometer and 80% acetone were poured in the cuvette (at least two-third) it was considered as blank. The Spectrophotometer was kept on constant at 0 absorbance for each wavelength (663 nm, 645 nm, and 510 nm). The blank was used to minimize the amount of 80% acetone in each concentration (control, 4%, 8% and 12%). The O.D of each concentration was carried out and calculated the amount of chlorophyll a, chlorophyll b and total chlorophyll by using the formula given by Arnon, (1949) and content of carotenoids was determined by the formula suggested by Harborne, (1973). Chlorophyll contents can be calculated by the following formula presented by Arnon, (1949).

$$\text{Chlorophyll "a"} \left(\frac{\text{mg}}{\text{g}}\right) = 12.7(A663) - 2.69\,(A645) \times (V|1000) \times W$$

$$\text{Chlorophyll "b"} \left(\frac{\text{mg}}{\text{g}}\right) = 22.9\,(A645) - 4.68\,(A663) \times (V|1000) \times W$$

$$\text{Total Chlorophyll} \left(\frac{\text{mg}}{\text{g}}\right) = 20.2\,(A645) + 8.02\,(A663) \times (V|1000) \times W$$

Where, A= Absorbance at specific wavelength, V= Final volume of Chlorophyll extract in 80% acetone, W= Fresh weight of tissue extracted

2.7.3. Determination of Carotenoids

The carotenes content can be calculated by following formula presented by Harborne, (1973).

$$\text{Amount of Carotenes in mg} = \frac{4 \times \text{O.D.} \times \text{Total volume of sample}}{\text{Weight of plant tissues}}$$

3. Results:

3.1. Petri-Plate Experiment

The Petri-Plate experiment was conducted in research lab of Department of Botany. The data of germination percentage was decreased when the concentration of extracts was increased, percent germination began to fall down 100% < 98% < 94% < 94%, respectively. The result of inhibition (%) illustrated that the maximum percent inhibition was observed in 12% extract while minimum percent inhibition was observed in 4% extracts as compared to control. The trend of percent inhibition shown as 0% < 2% < 6% < 6%. The data of germination index expressed that in control treatment the seeds were germinated with greater germination index but when concentrations of extract were increased, the germination index was gradually decreased as 94% < 81% < 63% < 60% (Table 1).

Table 1. Different germination parameters of *Vigna radiata* affected by *Helianthus annuus* in Petri-Plate experiment

Treatment	Different Parameters in Petri-Plate Experiment							
	1	2	3	4	5	6	7	8
Control	100%	94%	0	877.282	8.602±0.206	0	17.082±0.545	0
4%	98%	81%	2	672.69	6.754±0.802	21.48	10.79±0.24	36.78
8%	94%	63%	6	580.83	6.09±0.601	29.2	8.37±0.27	50.96
12%	94%	60%	6	398.614	4.15±0.224	51.7	8.13±0.29	52.35

1= Germination (%), **2=** Germination Index, **3=** Inhibition (%) in germination (%), **4=** S.V.I, **5=** Plumule length, **6=** Inhibition (%) in plumule length, **7=** Radicle length, and **8=** Inhibition (%) in radicle length

The observed data recorded on S.V.I. are interpreted that the minimum value was observed in 12% extract as compared to control 4% extract and 8%. The S.V.I. value was decreased as the strength of extracts were increased. This decline trend was obtained as 877.282 < 672.69< 580.836< 398.614. The data of radicle length was observed that the highest reduction was noted in radicle length in 12% extract as compared to control while the lowest reduction was obtained in 4% extract as compared to control. This reduction showed that *H. annuus* inhibit the radicle length as the strength of extracts were increased. The trend was observed as 17.08 <10.79 < 8.37 < 8.13cm. The observations sustained that the maximum rate of inhibition was obtained in 12% extract as compared to control, 4 and 8%. The result showed the trend as 0 % < 36.78 % < 50.96 % < 52.35%. The data of plumule length manifested that the length of plumule was significantly decreased as the concentrations of extract were increased. The maximum reduction in plumule length was observed in 12% extract as compared to control, 4 and 8%. The reduction trend showed as 8.60cm < 6.75cm< 6.09cm < 4.15cm. The data analysis annotated that the maximum 51.7% percent inhibition was obtained in plumule length in 12% as compared to control (0%) while minimum 21.4% percent inhibition was recognized in 4% and in 8% (Table 1).

3.2. Pot Experiment

The pot experiment was conducted in green house of Department of Botany. The data of germination was observed maximum germination percentage in control while in treatments as the amount of powder was increased, the germination percentage was decreased as in 4, 8, and slightly reduction in 12g powder. The decreasing trend shown as 98 < 92 < 90 < 90%. The highest inhibition in germinated seeds was found in 12 g powder extract as compared to control, 4, and 8 g powder extract. The trend of inhibition shown as 0% < 6.12% < 8.16 % < 8.16%. The data of germination index demonstrated that the germination index was affected as the amount of powder extract increased. The seeds germinated with greatest rate index in control while in 12g powder extract, seeds germinated with lowest rate index. The declined bias shown as 65% < 62% < 59% <38% (Table 2).

Table 2. Different growth parameters of *Vigna radiata* affected by *Helianthus annuus* in pot experiment.

Treatments	Different Parameters in Pot Experiment						
	1	2	3	4	5	6	7
Control	98%	65%	0 %	19.66±0.898	0	5.097±0.57	1.94±0.10
4g	92%	62%	6.12%	17.29±0.94	12.05	4.47±0.24	1.73±0.14
8g	90%	59%	8.16%	16.92±0.42	13.93	4.41±0.28	0.93±0.1
12g	90%	38%	8.16%	15.3±0.678	22.07	4.18±0.60	0.83±0.1

1= Germination (%), **2=** Germination Index, **3=** Inhibition (%) in germination (%), **4=** Plant height, **5=** Inhibition (%) in plant height, **6=** Fresh weight (g), **7=** Dry Weight (g)

The facts substantiated that there was significant decrement scrutinized in heights of 12 g potted plants**,** while in the other pots as the amount of powder extract increased the plant height slightly decreased in 8 and 4g potted plants as compared to control. The decrement course was obtained as 19.6<17.2<16.9<15.3. The compilations regarded that there is a remarkable inhibition found in the treated plants of 12g powder extract due to greater amount of powder as compared to control plants. The trend shown as 0< 12.0< 13.93<22.07. The fresh weight was found greater 5.09 g in control whereas lowest 4.18g fresh weight was obtained in 12g powder extract. Minimum 0.83 g dry weight was recognized in 12 g powder extract as compared to control 1.94g. The facts and figures demonstrated that there is declined bias archived in fresh weight and dry weight of the treated plants (Table 2).

3.3. Chlorophyll Content:

The data of chlorophyll contents and carotenoids computed in Table 3 illuminated that powder extract in high 12g amount caused reduction of chlorophylls and carotenoids as compared to control.

Table 3. Chlorophyll contents of *Vigna radiata* affected by *Helianthus annuus*

Treatment	Chlorophyll (a) mg/g	Chlorophyll (b) mg/g	Total Chlorophyll mg/g	Carotene
Control	0.046	0.073	0.120	14.2
4g	0.020	0.062	0.08	11.8
8g	0.014	0.045	0.059	8.8
12g	0.0056	0.029	0.035	5.8

4. Discussion

Hussain *et al.* (2004), Hussain and Ilahi, (2009), Samreen *et al.* (2009) stated that allelopathic substances released by plants are incorporated in soil and produced detrimental effects in the fields. Germination of seedling is the first crucial stage for growth and development of the plant. At this stage different metabolic changes take place which is necessary for the growth of the plant. It is indicated from our results that the different strengths of extract caused inhibition in the germination of seedlings of our test crop. There was inhibition in germination was pronounced in the treatments of *V. radiata* when plant extracts of *H. annuus* was incorporated. germination of seeds was reduced at higher concentrations 12% of extract. These results are in accordance with the findings of Kamal and Bano, (2008) who demonstrated that germination of seedlings of wheat was reduced due to aqueous extract of Sunflower. These results also correlated with the results of Khaliq *et al.* (2009) who reported that water extract of Sunflower significantly suppressed the germination of *Cichorium intybus.* The present results also associated with the dissertations of Ashrafi *et al.* (2008) who illustrated that significant inhibition was pronounced in the seed germination of wild barley caused by the application of sunflower extracts. Our results supported with the results of Mubeen *et al*. (2012) who elucidate that sunflower extract has inhibitory effects on germination index of *Dactyloctenium aegyptium.*

Our results supported with the findings of Ahmed e*t al.* (1995) who stated that the aqueous or powder extract of *H. annuus* have significant inhibitory effect on germination and seedling growth of Cotton and other plants. In our research studies the radicle and plumule length of *Vigna radiata* was reduced as the extract was applied at greatest strength. Our results justified by the findings of Anderson *et al.* (1978) who documented that radicle and plumule elongation was inhibited due to the whole plant extract of sunflower. Our estimations were not interacting with the determination of Chung and Miller,

(1995) and Turk and Tawaha, (2002) who annotated that radicle growth was much more affected than shoot length due to the extracts of allelopathic plants. Our present results agree with the reports of Brummette and Burns, (1972) reported that phytotoxins present in sunflower residues also inhibit the growth and development of other crops. In present study, fresh weights, and dry weights of treated plants of *V. radiata* decreased as the amount of sunflower powder was associated in higher amount. These results correlate with Anjum and Bajwa, (2005) who demonstrated that the reduction in dry weight of *Phalaris minor, Chenopodium album, Coronopis didymus, Rumex dentatus* and *Medicago polymorpha* was observed due to the roots, leaves and stem extracts of Sunflower.

Another most important parameter of plant growth is chlorophyll content. Chlorophyll contents are the essential pigments in growth of plants, as they provide basic framework in photosynthesis. The amount of chlorophyll contents destroyed under stress conditions which tremendously affected the metabolic processes of plants. Farhoudi and Lee, (2012) documented that chlorophyll content of *Lolium* spp. as well as *Avena ludoviciana* seedlings reduced due to the application of safflower extract. These results supported our estimations in which the chlorophyll contents were inhibited due to the residues of sunflower. Our results are also in agreement of consistence of Stupnicka-Rodyznkiewicz *et al.* (2006) who elucidate that chlorophyll contents and photosynthetic rate were affected by allelochemicals released by plants residues.

5. Conclusion

H. annuus is an important oil seed crop which is also used in the field of agricultural sciences. In this present research, the allelopathic effect of *H. annuus* were determined on test crop *V. radiata.* It is economically important test crop which is cultivated in large scale. Therefore, it is selected to examine the bioassay investigation of the allelopathic potential of *H. annuus* against *V. radiata*. It has not been previously documented. In our present research, there were differential effects of powder extract of *H. annuus* was distinguished on different growth parameters like percent germination, germination index, radicle and plumule elongation, seedlings height, fresh weight, and dry weight and chlorophyll contents of test crop. During our research study, it was observed that when the sunflower powder extract applied to test crop., the extract showed inhibitory effects on different growth parameters which all were essential for plant growth. It is concluded that sunflower powder extract has allelopathic potential to inhibit the essential growth parameters of cultivated crop.

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Production and Synthesis of Bacterial Cellulose and Its Applications: A Review

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Abstract

The bacterial cellulose is produced by various bacteria, has proven itself versatile biomaterial as compared to the plant cellulose. Bacterial cellulose is gaining attention day by day because researchers are focusing on low-cost preparation and production of cellulose in high quality as well as quantity. This review article represents all information about unique structure, fermentative production, sources, factors, purification, and some biomedical and industrial applications of bacterial cellulose.

Keywords: Cellulose; Bacteria; Synthesis; *Gluconacetobacter xylinus*

1. Introduction:

The cellulose is cheap, insoluble, and abundantly found polysaccharide on earth, which we can get from plants or their waste and shows tremendous economic importance in various fields since its discovery. Plants produce much cellulose, and some bacteria also have ability to produce cellulose (Esa *et al.,* 2014). Demand of plant cellulose is maximizing day by day and due to it, wood consumption is

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also maximizing which is becoming responsible for global warming and removal of trees (Park *et al.,* 2003). After treatment with harsh alkali and acid, cellulose can branch with hemicelluloses and lignin to get product in pure form (Sun, 2008).

Bacterial cellulose (BC) is an effective carbohydrate polymer from its discovery in several fields. Cellulose produced by some bacteria is much pure than plant cellulose, with better properties in structure and mechanical features (Ul-Islam *et al.,* 2012a; 2012b). The cellulose which we get from bacteria has highest purity and a reason to attract researchers and many industrial sectors (Gorgieva and Trček, 2019). BC contains glucose monomer so it has great properties and unique structure (Chen *et al.,* 2010), it can hold higher amount of water (Saibuatong and Phisalaphong, 2010). BC have higher properties of crystallinity (Keshk, 2014). It has also high polymerization and mechanical strength (Dahman *et al.,* 2010; Castro *et al.,* 2011). *Acetobacter, Rhizobium, Agrobacterium* and *Sarcina* are different genera of bacteria that can synthesize cellulose. BC is logically yield by a Gram negative and acetic acid bacterium known as *Gluconacetobacter xylinus*. It is mostly used to produce BC (El-Saied *et al.,* 2004), it yields cellulose in the liquid medium at high level and assimilate different sugars (Sani and Dalman, 2010; Moosavi-Nasab and Yousefi, 2011). Brown, (1886) initially reported the BC when working on Bacterium aceti in 1886 and observed Acetobacter that also producing cellulose in existence of oxygen and also glucose (Chawla *et al.,* 2009).

In industries to attain cellulose in high demand, BC production was anticipated. On land, Cellulose manufacture in plant cells done by photosynthesis. In oceans, algae or unicellular plankton also manufacture cellulose by using same kind of fixation of carbon dioxide used by land plants during photosynthesis. These organisms are substantial resource of cellulose production. Various photosynthetic microbes can fabricate cellulose (Keshk, 2014). Many review articles related to the structure and production of cellulose have appeared. This review reported the structure, cultivation modes, factors that effect on BC production, biosynthesis of bacterial cellulose by *Gluconacetobacter xylinus*, its sources, purification by several methods as well as applications of bacterial cellulose in different industries. BC is economically beneficial for us so; its production should be done at high level and low cost.

1.1. Objectives:

- To explore the study of production and synthesis of bacterial cellulose and its applications.
- To determine, the sources and structure of bacterial cellulose.
- To find out the new advance horizons of bacterial cellulose activities.

2. Structure of Bacterial Cellulose:

The BC made up of fibrils and contain β 1 to 4 linked glucose units in chain form with molecular formula ($C_6H_{10}O_5$). This chain joined together by hydrogen binding (Ul-Islam *et al.,* 2012a) (Figure 1). Researchers revealed that plant cellulose and bacterial cellulose are identical to each other chemically but different from each other structurally. BC chains unite together to form subfibrils which crystallized and form microfibrils (Jonas and Farah, 1998), then microfibrils into bundles, and then into the ribbons (Yamanaka and Sugiyama, 2000). BC become highly porous because its fibrils are of approximately 100 times thinner as compared to plant cellulose (Chawla *et al.,* 2009; Gayathry and Gopalaswamy, 2014).

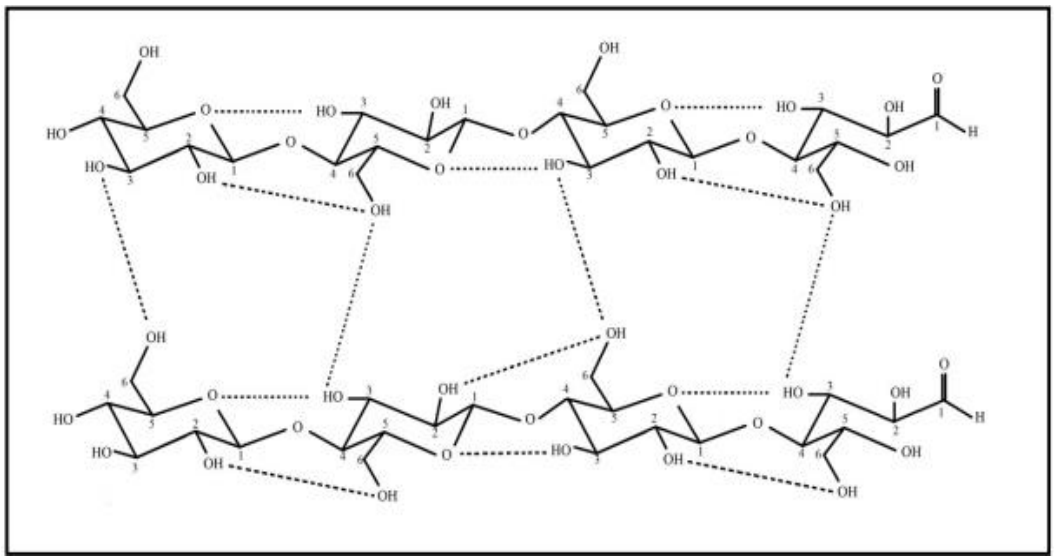

Figure 1. Hydrogen bonding in Bacterial cellulose (Festucci-Buselli *et al.,* 2007).

Chemically, bacterial cellulose (BC) show resemblance to the plant cellulose (PC) but it has difference in structure and properties (Figure 2). BC are the thinnest naturally occurring fibers which have 1.5nm width (Iguchi *et al.,* 2000).

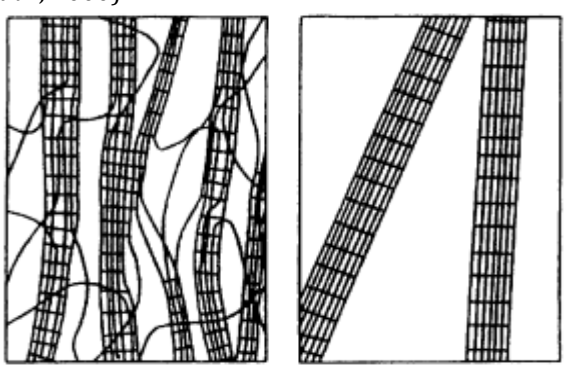

Figure 2. Comparison of BC microfibrils with fibrils of Plant Cellulose (Iguchi *et al.,* 2000)**.**

2.1. Sources

The wood and cotton together are the most important resources of cellulose production for products of cellulose i.e., textiles, paper, cardboard and construction materials and cellulose derivatives as cellulose acetate, cellophane, and rayon (Keshk, 2014). Genera *Gluconacetobacter, Salmonella, Agrobacterium, Azotobacter, Achromobacter, Rhizobium, Aerobacter* and *Sarcina* are those species of bacteria that can synthesized cellulose (Shoda and Sugano *et al.,* 2005). Major source of BC is *G. xylinus*. Cellulose synthesized by both Gram positive such as *Sarcina ventriculi* and Gram-negative bacteria *Rhizobium, Agrobacterium, Salmonella, Achromobacter, Azotobacter, Pseudomonas, Aerobacter, Sarcina, Acetobacter, Dickeya, Rhodobacter* and *Alcaligenes*. *A. xylinum (G. xylinus), A. hansenii* and *A. pasteurianus* are most active producers of cellulose (Brown, 2004; Chawla *et al.,* 2009; Jahn *et al.,* 2011;

Morgan *et al.,* 2013). Some microorganisms such as *Salmonella* and *E. coli* species also produced cellulose, which show multicellular behaviour (Zogaj *et al.,* 2001). Cellulose synthesis genes of these species show resemblance to the *G. xylinus* (Solano *et al.,* 2002).

2.2. Biosynthesis of Bacterial Cellulose by G. xylinus:

The Synthesis of bacterial cellulose from *G. xylinus* depend on physiological state of cell and also on the Krebs cycle or on the cycle of pentose phosphate which make pair with glyconeogenesis. Regulation of bacterial cellulose is a process of many steps which involve large amount of enzymes, proteins, catalytic complexes, and their molecular structure is not clearly characterized (Pokalwar *et al.,* 2010). Pathway of cellulose was suggested by Colvin and Leppard, (1977), in this pathway glucose show reaction and in the existence of GK form glucose-6- phosphate. Glucose-6-phosphate form glucose-1-phosphate and then into UDP-glucose (uridine diphosphoglucose) and finally cellulose formed (Lin *et al.,* 2013).

Biosynthetic mechanism of cellulose occurs in the presence of various key enzymes such as glucokinase, isomerase, fructose-1-phosphate kinase, UDPG-pyrophosphorylase, cellulose synthase and phosphoglucomutase. It includes hexose monophosphate pathway (HMP) and Krebs cycle (Yu *et al.,* 2012). This system was initially reported by Glaser (1958), he reported that synthesis of cellulose in vitro only gets from UDPG, some broken cells and ATP. *G. xylinus* converted some carbon compounds into the cellulose (Figure 3). These carbon compounds are glycerol, pyruvate, dihydroxyacetone, and Hexoses. Dicarboxylic acids and Pyruvate when incorporate into Krebs cycle then because of oxalacetate decarboxylation, some changes take place in the pyruvate and it is converted into pyruvate hexoses through gluconeogenesis (Chawla *et al.,* 2009).

Moreover, the activity of pyrophosphorylase enzyme in this pathway is different in different *G. xylinus* strains while the effective cellulose producer is *G. xylinus* spp. sucrofermentans BPR2001 (Bielecki *et al.,* 2005).

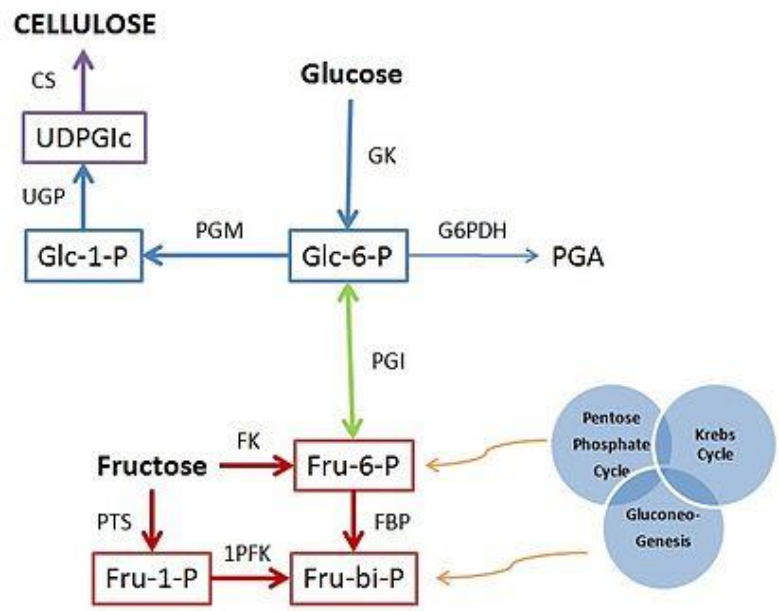

Figure 3. Pathway of cellulose synthesis by *G. xylinus Source: (Source: https://en.wikipedia.org/wiki/Bacterial_cellulose#/media/File:Biochemical_Pathway_for_Cellulose_Synthesis.jpg*)

2.3. Production methods

Bacterial cellulose has been normally produced by several methods which are known as static culture method (Kralisch *et al.,* 2010; Shezad *et al.,* 2010) and agitated culture method (Yan *et al.,* 2008; Tse *et al.,* 2010) and by using different reactors (Song *et al.,* 2009.

2.4. Agitated Culture (Submerged Fermentation)

The strain that is producing bacterial cellulose turn the BC into cellulose-negative mutants by agitated culture method, by this it more enhances the quality of strain due to its rapid growth, thereby also lowering BC productivity. The limitations of static cultures (diffusion, controllability, and scale-up) can be overcome through switching to submerged production. Static culture system because of its low yield cause problem to retard the industrial application of bacterial cellulose. Cellulose can be economical mass produced utilizing agitated culture system. For bacterial cellulose production, the most acceptable organic nitrogen source was found corn steep liquor. In corn steep liquor lactate is present which invigorating bacterial cellulose production and cell growth. Each fermentation has its own advantage, but Batch fermentation may be adequate for producing bacterial cellulose in the form of pellicle for bulk applications and Fed-batch fermentation or continuous fermentation may be advantageous particularly to produce modified or composite bacterial cellulose pellicles. On the production of bacterial cellulose on the large scale, a major difficulty comes in the successful commercialization of the technology that is the lack of available biochemical engineering knowledge. The product of this system has not pellicle form, although it is formed in reticulated cellulose granules (Pokalwar *et al.,* 2010).

Agitated culture synthesize snow- like, fibrous and rice- like cellulose. Mostly advantages of bacterial cellulose are of misplaced if the fibers are not organized into a defined pellicle, and applications for this product have been limited (Yan *et al.,* 2008). Improper shape of SCP (sphere like cellulose particles) produced in both Stirred and agitated conditions while it was reported that 3D reticular pellicles produced only in static condition (Tanskul *et al.,* 2013). Agitated condition is also responsible for the production of SCP (Wu and Lia, 2008; Yan *et al.,* 2008). The SCP has lower polymerization, mechanical strength and the crystallinity from that pellicle which is produced from static culture (Shi *et al.,* 2013).

2.5. Static Culture

The Static culture method is widely used method for the cellulose production from *G. xylinus* at the air or liquid compound but gives low productivity because of manpower and longish culture period; it is also a simple, low tech and traditional method of cellulose production which is inhibited by mixing and aeration. Regulation of Bacterial cellulose production from medium surface in static culture is done by air supply and its production depends on some concentration of carbon source moderately. With the increase in time of growth the production of BC with C-H and hydrogen bonding also increase (Sheykhnazari *et al.,* 2011). When all bacteria snare and growth of pellicle become downward then bacterial cellulose synthesis reach its limit. If amount of oxygen is inadequate for bacteria, then they become inactive. BC productivity increase in semi-continuous in contrast with the continuous process at industrial level (Çakar *et al.,* 2014). Static culture gives lower yield of BC production that's why for the commercial purpose cellulose mostly produced from agitated fermentation. Disruption effects of aeration on the H-bonds formation between the cellulose was proposed by altered microfibrils organization (Bootten *et al.,* 2008). Hu *et al.* (2013) found that concentration of initial glucose causes

an effect on the SCP mean diameter and when volume of inoculums increases then number of SCP also decreased. So, the formation method of SCP is still unknown (Chawla *et al.,* 2009).

3. Bacterial Cellulose Production by using reactors:

The BC produced by agitated and static culture method. But for the production of BC such type of reactors development is necessary which are different from agitated and static culture method. In agitated culture conversion of BC strain occur into cellulose negative mutants while static culture takes much time but low productivity, reactors do not allow these.

3.1. Rotating Biofilm Contactor (RBC)

In this reactor many circular disks are present in series along with a horizontal shaft. When rotation of disks occurs in RBC then it also exposed with the medium and air. BC strains do not face a strong shear stress during cultivation in the RBC and can easily transfer oxygen from air. *G. xylinus* species produce in RBC by Kim *et al.* (2007). In RBC reactor aeration rate for *G. xylinus* is set on 1.25 vvm and give production of 5.67 gram per liter while with the use of eight disks it gives maximum BC production of about 5.52 gram per liter. The impeller rotates in RBC with 15 rpm speed (Chawla *et al.,* 2009).

3.2. Spin Filter Reactor

This reactor was firstly developed by Jung *et al.* (2007). The reactor contains six impellers with blades, spin filter, cylinder covered by stainless steel units, agitated shaft with steel bottom. BC can be produced by this reactor from by *G. hansenii* PJK. BC production by this reactor is 4.57 gram per liter after 140 hours of cultivation by which we can obtain 2.9 times higher than by jar fermentor (Chawla *et al.,* 2009).

3.3. Rotating Disk Reactor (RDR)

In this reactor a rotating disk is present which is immersed half in the medium broth and the other half is in the atmosphere. Disk rotates continuously to retrieve oxygen from atmosphere and nutrients from the medium (Chawla *et al.,* 2009). In rotating disk reactor optimum conditions for BC production is explored by Krystynowicz *et al.* (2002). It is done by some changes in the no. of disks, rotation speed of reactor and volume of medium. In this reactor BC production occur in high amount when rotation speed was 4 rpm and the ratio were 0.71 cm-1 from surface area to the medium volume (S/V) (Shoda and Sugano, 2005).

3.4. Silicone Membrane Reactor

This reactor is used for BC production from *A. pasteurianus,* a system was developed by Yoshino *et al.* (1996). In this system liquid surface and a synthetic membrane which is oxygen-permeable is used and cellulose pellicles can obtain. By use of cylindrical vessel BC production become double. In this reactor a silicon air bag is present to produce BC. Production of bacterial cellulose on silicon membrane is completely dependent on roughness of membrane surface, so it gives five times higher production of BC.

3.5. Modified Airlift Reactor

For BC production airlift bioreactors are also used, it requires low power supply as compared to the agitated bioreactor. Air supplies occur from the bottom and this oxygen enriched air is circulated in the medium. Chao *et al.* (2000) got 3.8 gram per Liter BC production after 67 hours of cultivation. Cheng *et al.* (2002) developed modified airlift reactor in which 3–net draft tubes are available and give 7.7 gram per Liter BC production after 72 hours of cultivation. By usage of airlift reactors, the maximum BC concentration was 10.4 gram per liter with a 0.22 g/l/h rate of production (Chao *et al.,* 2001b).

4. Factor affecting on BC production:

The factors that effect on cellulose production normally involve growth medium, product formation, and environmental conditions. Factors that effect on the production of cellulose are some environmental factors and growth medium that contain carbon, nitrogen, and nutrients.

4.1. Effect of medium components

The Organisms grow in such type of medium which contain nitrogen, carbon, and nutrients. If these components of the medium change, then it causes major effect on the growth of microorganism and formation of product directly or indirectly. Abundance of carbon source and minimal of nitrogen source in medium for bacteria secretes most noticeable exopolysaccharides. Sometimes to increase the growth of cell and product formation.

4.2. Effect of precursors

The precursor molecules are also important during synthesis of polysaccharide. Some researchers observed that amino acids are used as stimulator for improving the yield of biopolymer and also as nitrogen source (Son *et al.,* 2003). Methionine is an amino acid effect on production of BC by species of *Acetobacter xylinum*, Sucrofermentans, and cause 90 % growth of cell and production of cellulose. Nicotinamide is also important for production of cellulose, its amount from 0.00001 to 0.00008 % showed that at the range of 0.00005% maximum production of bacterial cellulose occur. Some vitamins show contradictory effects such as pantothenate and riboflavin while some vitamins such as biotin, nicotinic acid, pyridoxine and p-aminobenzoic acid are also important for growth of cells and production of cellulose. Sugar nucleotides also show important effects on growth of cells and production of cellulose, UDPGlc which act as important precursor in the bacterial cellulose production as a stimulator (Bae *et al.,* 2004).

4.3. Effect of water-soluble polysaccharides

Culture that is used in a reactor for BC production is complex because it contains liquid medium, air gas and solid BC and cells. When BC coagulates then cells adhere and, on the surface, a large clump formed and subsequent desolation of cellulose production. For sufficient transfer of oxygen and enough amount of agitation small and homogeneous bacterial cellulose suspension is necessary. To reduce the Clumps that are formed at the surface water-soluble polysaccharide added to medium. Production of BC in airlift reactor increase by the addition of 0.1% agar in 50 L (Chao *et al.,* 2001a). At the agar fraction of 0.4% production of BC enhanced from 8-12.8 grams/L. It is suggested that agar prevent the coagulation of BC in broth and amount of free cell numbers increase because of this

property (Chawla *et al.,* 2009).

4.4. Effect of environmental factors.

The environmental factors effect on the microorganisms rapidly, it changes the cell morphology and induction. These environmental parameters are pH, temperature, dissolved oxygen, and stirrer speed.

4.4.1. Carbon source

For the production of bacterial cellulose expensive synthetic medias are required. So, to reduce BC production cost some alternative sources of natural carbon are used i.e., waste material from food industry (Gorgieva and Trček, 2019). Mostly used carbon sources for cellulose synthesis are glucose and sucrose while some other sources have also been tried as carbon source i.e., starch, xylose, maltose, fructose and maltose. By using glucose as carbon source, 1.72 g/L cellulose produced from *G. hansenii* PJK (Park *et al.,* 2003) same as by using glucose as carbon source, 4.16 g/L produced from Acetobacter sp. V6 strain, and these strains are isolated from fermented vinegar (Son *et al.,* 2003). Initial concentration of glucose is important for cellulose production but gluconic acid which formed in medium as by product reduce the cellulose production and culture pH. Carbon source such as xylose is used for the production of cellulose by *G. xylinus* (Ishihara *et al.,* 2002). For the cellulose production from *G. xylinus* NCIM 2526 Sucrose, mannitol and glucose are used as carbon source (Ramana *et al.,* 2000). Mannitol as carbon source when combine with the *G. xylinus* from kombucha then it produces high amount of cellulose (Nguyen *et al.,* 2008). Ethanol as carbon source also effect on the production of cellulose in *G. hansenii*, increase the yield from 1.30 to 2.31 gram per Liter (Park *et al.,* 2003). *Acetobacter* species A9 strain are used for cellulose production and (Son *et al.,* 2001) observe the effect of ethanol by adding 1.4% ethanol in the medium, this addition increases the cellulose production four times than before, it produces about 15.2 g/L cellulose. Cells that cannot produce cellulose also eliminate by adding ethanol. Gluconic acid is formed in the medium and it reduce the pH and cellulose production if its carbon source is glucose.

Keshk and Sameshima, (2006) observed that in presence of lignosulphonate formation of gluconic acid and BC, by this addition of lignosulphonate production of gluconic acid decrease while BC production increase. In polyphenolic and lignosulphonate antioxidant compounds are important to inhibit the formation of gluconic acid.

4.4.2. Nitrogen source

The nitrogen sources also effects BC production such as if amount of nitrogen exceed then it favors the production of biomass but reduce the production of cellulose. Casein hydrolyzate produces about 5 g/L while peptone produce about 4.8 gram per Liter in *G. xylinus* (Ramana *et al.,* 2000). Corn steep liquor is used as nitrogen source, and it also effect on cellulose production when 0.15% corn steep liquor with 4% fructose is added to the medium. Corn steep liquor is a good nitrogen source because it contains lactate which is normally not present in other nitrogen sources. Yeast extract concentration also effect on growth of cell and cellulose production. If carbon source is 20 grams/L then add 5 to 60 grams/L yeast extract, by this 6.7 g/L cellulose can be produced when medium contain 40 grams/L of yeast extract (Chawla *et al.,* 2009).

4.4.3. Temperature

The temperature is also an important parameter and show effect on the production of cellulose and growth of cell. The maximum production of cellulose by various experiments was observed at the temperature between 28 and 30°C (Chawla *et al.,* 2009).

4.4.4. pH effect

The pH effect is mostly studied by Hutchens *et al.* (2007). During the static fermentation pH comes to 3.5 which is beyond the optimal range of PH for production of BC (Klemm *et al.,* 2001). The pH control is much important for the production of BC. Embuscado *et al.* (1994) reported that cellulose production takes place at 4.5 which is its optimal pH, cellulose is not synthesized when pH is below from 3.5. For this purpose, Hwang *et al.* (1999) proposed a pH shifting method. They reported that achievement of BC production is possible by shifting of pH from 4.0 to 5.5 in batch culture because when pH was 4.0 *G. xylinus* start to accumulate gluconic acid. Hutchens *et al.* (2007) reported that initial pH was 5.5 when bacteria was cultured on glucose but when mannitol was used then optimal pH was 6.5. Respiratory metabolism of *G. xylinus* is responsible for low pH of static culture under static batch cultivation because ethanol oxidation is converted into acetic acid and glucose is converted into gluconic acid. For the growth of cell and production of cellulose control of pH is much important (Kongruang 2008; *Ha et al.,* 2011). Moreover, pH remains constant in the static culture because of periodic additive fresh medium (Shezad *et al.,* 2010). PH effect on thickness of BC which is produced in static fermentation by coconut water medium was studied by Jagannath *et al.* (2008).

4.4.5. Dissolved oxygen.

In the production of cellulose dissolved oxygen play very important role. For the synthesis of cellulose and secretion, aerobic condition is required. *G. xylinus* produces a viewable film of pellicle of cellulose in static culture which mostly covers the surface of medium (Ross *et al.,* 1991). During production of BC critical concentration of dissolved oxygen should be kept on more than 3.10 ppm for prevention of oxygen limitation. At low shear force, synthesis of BC by the *Gluconacetobacter* sp. contain much oxygen transfer rate (Lin *et al.,* 2013). Capacity of dissolved oxygen can be increased in the medium with the changing speed of agitation. Tantratian *et al.* (2005) studied that if the amount of oxygen in the medium is high and glucose is used as carbon source then gluconic acid content also increase, but if dissolved oxygen is too low then cellulose production again decreases because of less oxygen (Chawla *et al.* 2009).

4.4.6. Purification

Cellulose which we get from bacteria after fermentation includes some cells and some medium components in the form of impurities. During crude products of such types of yield, it also contains impurities of cells and bounded to polymer and so it resumed from broth of fermentation (Bieleck *et al.,* 2005). To get cellulose in pure form fermented broth should be purified. The process by which BC purification occur in culture medium are treated with organic acids such as acetic acid and alkali i.e. (KOH or NAOH), or it can also be done by continual washing of mixtures with hot tap water or with the reverse osmosis water for some period of time. This method of purification occurs alone and also in combined form. Cells that are trapped in Bacterial cellulose can be removed to purify it by treated them with solutions such as NaOH or KOH or Na2CO3 at temperature 100 °C for approximately 15 to 20 min.

After that, impurities can be removed to filter the solution by using an aspirator. Apply the distilled water repeatedly on the filter cake till its pH became neutral. Without any other microbes in dry mass of BC it can be measured after drying for 4-6 hours. By doing this difference occur between those BC which is treated by NaOH and the mass of dried BC having cells (Jung *et al.,* 2005; Kongruang, 2008).

After addition of NaOH solution the culture medium can be treated with acetic acid. After that treat again with the distilled water to neutralize its PH (Keshk *et al.,* 2005). For Medical application, removal of toxins which are responsible for causing pyrogenic reactions and removal of some bacterial cells occur in BC by specific method. Process the cellulose between sheets of absorbent to remove its 80% liquid phase. So, repeat this process three times then incubate in 3% solution of HCL, pressed gently and washed with distilled water. Purified pellicle which Sterilized in autoclave mostly give excellent performance as wound dressing (Chawla *et al.,* 2009).

5. Applications of BC

The Bacterial cellulose has a so many applications in biomedical area (Azeredo *et al.,* 2019). BC have many applications in various fields such as artificial blood vessels and artificial skin, tissue engineering (Rajwade *et al.,* 2015; Tsouko *et al.,* 2015), paper restoration, regeneration of blood vessels (Wippermann *et al.,* 2009), dressing of wounds (Muangman *et al.,* 2011), food industries, textile industry, pharmaceutical, mining, refinery (Shah and Brown, 2005; Czaja *et al.,* 2006), cosmetics and medicines (Chawla *et al.,* 2009). White and brown (1989) initially reported that microbial cellulose can use for the purpose of commercialization. Because of wide applications of BC, number of BC patent and journals shows high trend (Siro and Plackett, 2010).

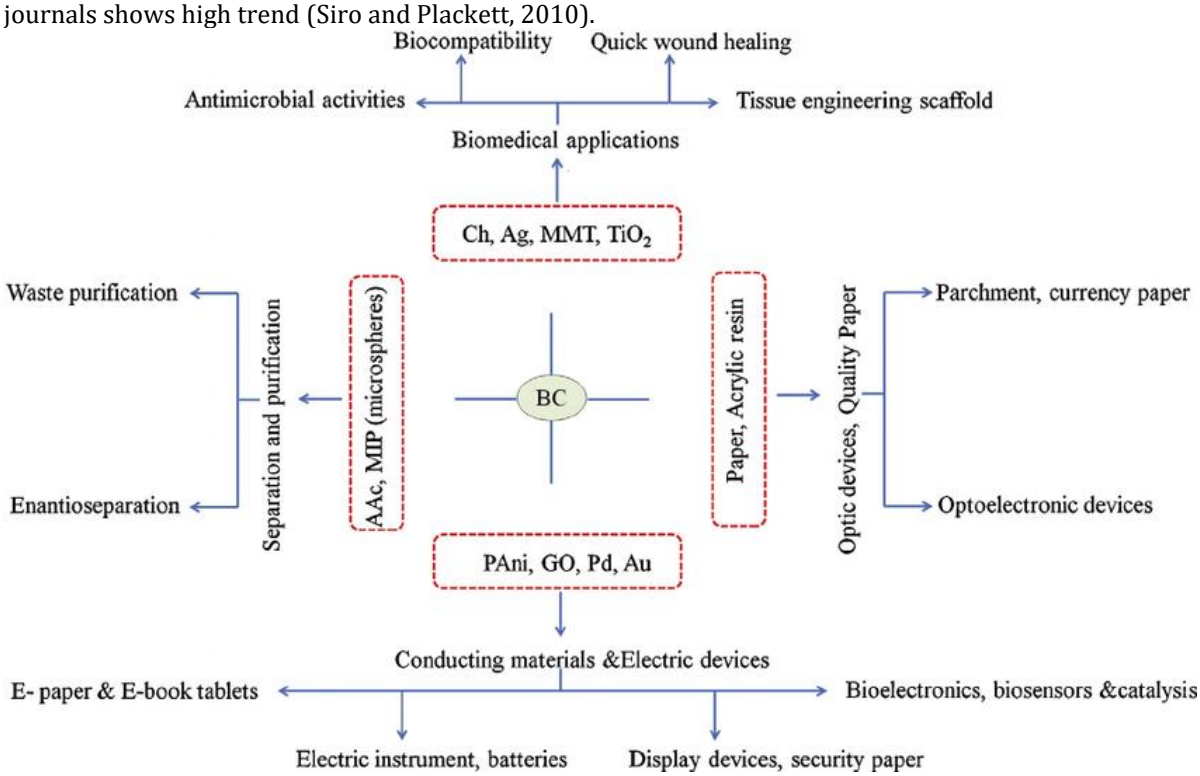

Figure 4. Illustration of BC with various materials for specified applications in different fields (Shah *et al.,* 2013).

5.1. Medical Application:

5.1.1. Wound Healing

The BC is mostly used in medical fields as antifungal, antimicrobial, biocompatibility in BC and tissue regeneration. In wound dressing material and tissue engineering scaffold BC composites show much better result (Islam *et al.,* 2020). BC-Ch composite has higher water holding capacity, retention of water for a long time, slow release of water and all properties are important for the wound healing (Ciechanska, 2004; Ul-Islam *et al.,* 2011). Wounds that are treated by BC-Ch composites can regenerate earlier than commercially available dressing material (Lin *et al.,* 2013). Prepared BC-gelatin composite has much better biocompatibility than the pure BC, also used as wound healing material and tissue engineering scaffold (Kim *et al.,* 2010). Result of this type of BC composites provides rapid and safe wound healing (Maneerung *et al.,* 2007; Maria *et al.,* 2010). Pure gelatinous membrane which is made up of BC is used as artificial skin (wound dressing) in Brazil (Keshk, 2014). Wound healing includes interaction of different cell types, soluble compounds, and extracellular matrix molecules (Eming *et al.,* 2002). Winter (1962) ascertained that if wound is in moist condition, then healing and re-epithelialization occur. BC has unique properties and are most effective for the wound healing purpose (Alvarez *et al.,* 2004; Legeza *et al.,* 2004; Czaja *et al.,* 2006; Czaja *et al.,* 2007; Stanislaw *et al.,* 2012).

5.1.2. Skin therapy

The BC have gelatinous membrane which is used as artificial skin to cover the wound temporary which is indicated by the good mechanical strength, and less skin irritation and significant permeability for the gases and liquids. Some products of BC i.e., Biofill and Gengiflex are used in dental implants, surgery and realities in care centers of human health. For human skin biofill work as temporary substitute and cause treatment of burns and ulcer successfully. Biofill is used in more than 300 treatments and some advantages of Biofill are that it gives relief from pain immediately, also useful in wound healing faster, decrease the infection, transparency, decrease the cost and time apply on treatment, improved exudates retention, and spontaneous detachment. A disadvantage is also mentioned that limited elasticity in areas of great mobility. Not only Biofill but also Gengliflex used to recover the damage tissues (Keshk, 2014). Many authors published applications and results of Biofill and Gengiflex. Schmauder *et al., 1996* reported that bacterial cellulose recently treated the large skin wounds of dogs and horses in veterinary medicine (Kawecki *et al.,* 2004).

5.1.3. Artificial blood vessels

The Bypass operation become necessary in blockage of coronary vessels due to hardening of the arteries present around the heart. Artificial blood vessels can be made by the bacterial cellulose, but it is little risky as compared to synthetic material which is used in bypass operation because of some blood clotting occur in artificial blood vessels. This proves that cellulose in contact with the blood can work properly and really well for the formation of artificial blood vessels. Those blood vessels that are real in the body contain an internal layer of cells that provide protection from blood clotting. Tube which forms from cellulose has 1mm inner diameter, 5mm length and 0.7 mm thickness of wall. BASYC tubes are used in microsurgery and these parameters are enough for the requirement of microsurgery. BC has significant mechanical strength which can handle into a film or sheet. Polyvinyl alcohol (PVA) has characteristics of biomedical applications. By using a method known as novel thermal processing under the applied strain, some amount of BC nano fibers can create PVA-BC nano composites. These

nano composites have mechanical properties, including anisotropy match with soft tissues mechanical properties, it can replace and ranges from cardiovascular tissues to the connective tissues (Keshk, 2014). Same as, nata de coco is used for production of bioethanol because of its effective cost and structural strength (Montealegre *et al.,* 2012). For the preservation and protection, Packaging of food material played an important role. Packing should be Bio-based in industries. BC identified as the suitable material (Tang *et al.,* 2012) because it contains biodegradable performance (Sonia and Dasan, 2013), much water resistance and fine network (Arrieta *et al.,* 2014).

5.1.4. Tablet modification

The *G. xylinus* BC and kenaf are used for the preparation of microcrystalline cellulose (Keshk and Haijia, 2011). Some developed bacterial cellulose (DBC) and developed kenaf cellulose (DKF) also show the properties of crystalline structure having 85% crystallinity in DBC and 70% crystallinity in DKF. Its particle size are larger (5-20 µm) than DBC (1-5 µm). Avicel PH 101 and DKF have less density than DBC and during binding and flow processes both show same behavior (Czaja *et al.,* 2006; Basta and El-Saied., 2009). Determination of thermal properties of Developed bacterial Cellulose and Developed Kenaf materials were done by thermo-gravimetric analysis (Gouda and Keshk, 2010).

Furthermore because of dissipation of cellulose, DBC loss weight by a single step of degradation process which is mostly 320°C to 380°C. Difference between thermal properties of cellulose material occurs because of crystallinity. DBC have 85 percent degree of crystallinity while DKF have 77% degree of crystallinity. DBC degrade at 320°C and DKF degrade at 215°C because a great crystalline structure degrades at high temperature (Keshk, 2014).

5.2. Industrial Applications

5.2.1. Food Industry

The Carbon source used for Acetobacter xylinum is coconut water which is then changes into extracellular cellulose (Esa *et al.,* 2014). BC can be synthesized by coconut water in static culture (Jagannath *et al.,* 2008). Nata de Coco is a desert of Philippine that is used by the Philippines over seventy years (Keshk and Sameshima, 2006). Nata is used as snack because of its soft texture. The Nata is also import from Philippine into the Japan for the expansion of cellulose production. Cellulose in pure form that can also use as thickening agent in food, cellulose can combine with intestinal tract of the human and because of its complete indigestibility used as food base. In 1970s, monacolin K (mevinolin) was identified as metabolite of the *Monascus* sp. and inhibit the cholesterol synthesis. During process of production, bacterial cellulose manufactured in static culture of coconut's water (Jagannath *et al.,* 2008). When nata de coco and cereal mixed with each other then they have ability to decrease lipid level in consumer (Mesomya *et al.,* 2006).

5.2.1.1. Replacement of Fat:

Fats play important role in textural and physical appearance of different foods. Fats are present in cakes to keep it soft and full of flavor (Rios *et al.,* 2018). Intake of too much fat is responsible for many heath issues such as diabetes, obesity, heart disease and increase in blood cholesterol; so, to reduce content of fat from food products much efforts was done (Guasch-Ferre *et al.,* 2015). Those who consume too much fats should have awareness of its harmful effects and have demanded to reduce fat products. There is a view that high fat food is much desirable as compared to low fat food. So, from last

some decades, researchers are working on such ingredients which are suitable in replacement of fats. The main purpose of fat replacement is to mimic it with less calorie product and to avoid health problems. Bacterial cellulose can be used as fat replacer in food (Azerado *et al.,* 2019).

5.2.2. Paper Industry

For the manufacturing of good quality paper bacterial cellulose from agitated culture is much suitable, it is reported by Johnson and neoji (1989). According to Watanabe and Yamanaka (1994), disintegrated BC in the paper pulp increase the tensile strength of paper and also increase its power of folding (Iguchi *et al.,* 2000). Only 15% addition of BC in pure pulp paper increase the folding capacity of paper than pure paper pulp. By the addition of BC elastic modulus increase from 2.0 to 3.5 (GPa). When 5% BC mixed with wood pulp for manufacturing of paper then it improves the fire resistance, strength and kaolin retention properties of paper (Basta and El-Saied, 2009).

6. Conclusion

The Bacterial Cellulose is attractive because of its unique structure, purity, stability, high amount of water, high polymerization, and excellent permeability. BC have ability to replace plants for cellulose production through static and agitated cultural methods. Use of several reactors i.e., Spin filter reactor, rotating disk reactor, rotating biofilm contractor, silicon membrane reactor, modified airlift reactor, is a useful solution of BC production. Researchers are focusing on high yielding and purification of bacterial cellulose in low cost. Work on genetic modification of bacteria is also an approach to increase BC production. Because of many reasons BC has commercial values in different fields and this increased value attract the researchers and industrialists. *G. xylinus* is a bacterium who has not faster growth rate as compared to other bacteria, but little bit genetic modification can increase its production with better quality. BC from modified organism can play a major role in medical, paper as well as food industry.

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GU JOURNAL OF PHYTOSCIENCES

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Applications of Alpha (α)-Amylase in Biotechnology: A Review

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Abstract

Alpha-amylase is an enzyme that hydrolyzes alpha-bonds into lengthy polysaccharides linked to the alpha bonds, such as starch and glycogen in order to form shorter chains, dextrin and maltose. 1, 4-alpha-D-gylcosidic linkage endohydrolysis is catalyzed by amylase, including 3 or more, 4-linked glucose units (E.C.3.2.1.1). The enzyme is utilized to free starches, glycogen and oligosaccharides from reduction groups. In numerous industries, this enzyme has several applications including food, textiles, bakery, pharmaceutical products, and starch processing and detergent products. With thebiotechnological Progression, the range of its applications in various sectors have increases including clinical sector, medicinal, and analytical chemistry.

Keywords: α-amylase; Enzyme; Applications; Biotechnology

1. Introduction:

Microbial enzymes, including alpha-amylase are the most significant industrial enzymes, are prevalent in industrial operations such as brewing, baking, textile, pharmaceutical products, stomach therapies and detergents etc. α- Amylases represent around 25% of the market in enzymes and are the most diverse enzymes in the industrial enzyme business (Sidhu *et al.,* 1997). A-amylase enzymes are included in animals, plants, and microorganisms (EC 3.2.1.1). Microorganisms are crucial for enzyme manufacturing because of their short growth cycle. α-amylase, with different action patterns, catalyses

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the hydrolysis of internal alpha-glucosidal connections between starch and other oligo- and polysaccharides. Amylase in the food and textile industries is commonly utilized (Yamamoto, 1995; Uhling, 1998). Amylases represent about 30 percent of the global production of enzymes as a form of industrial enzyme (Outtrup and Jorgensen, 2002). The products are used in a number of industries, including food, fermentation, textiles, paper, washing machines and sugar (Calik and Ozdamar, 2001). The following are classified as strong amylase producers, B subtilis, B. stearothermophilus, B. licheniformis and B. amyloliquefaciens and are extensively utilized for industrial enzyme processing on a varying scale for applications (Sivaramakrishnan *et al.*, 2006). In the starch manufacturing business, microbial amylases nearly replace chemical starch hydrolysis (Veille and Zeikus, 2001). Recombinant amylase may have been transferred from -amylase genes to various microbial hosts through the most popular genetic engineering approach (Karakas *et al.,* 2010).

1.1. Objectives:

- To explore the new advance applications of Biotechnology.
- To determine the new horizons of α-amylase and its application in Biological Sciences.

2. Mechanism of Alpha (α)-amylase:

Alpha-amylase enzyme hydrolyses alpha bonds to create shorter chains, dextrin, and maltose in long, Alpha-like polysaccharides, such starch and glycogen. The endo-hydrolysis of 1 and 4-alpho-D-glucose links is catalysed by alpha-amylase in polysaccharides containing 3 or more 1 and is 4-linked (Figure 1). The mechanism consists of a twofold substitution mechanism that catalyses the substratum hydrolysis, generating an intermediate covalent glycosyl enzyme hydrolyzed by transitional oxocarbenium-like states (Sivaramakrishnan *et al.*, 2006).

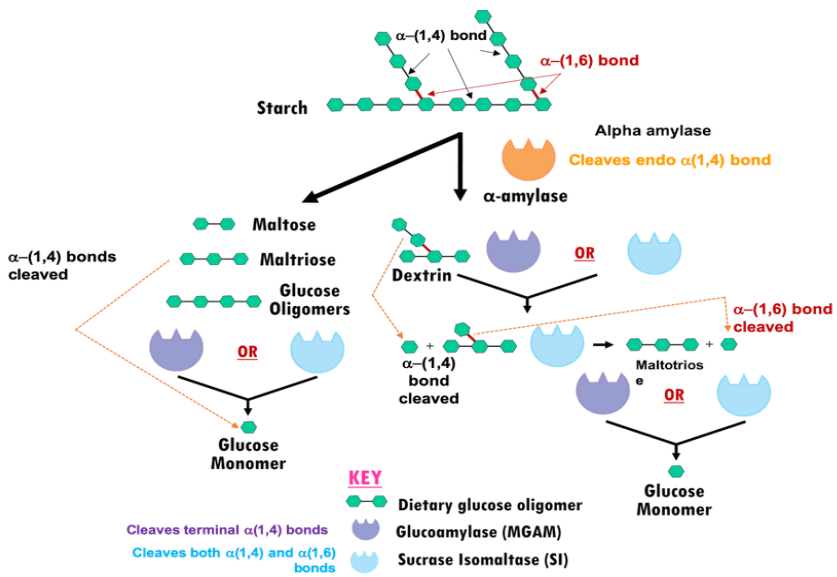

Figure 1. Source: https://pdb101.rcsb.org/global-health/diabetes-mellitus/drugs/alpha-glucosidase-inhibitors/alpha-glucosidase

3. Amylase producing organisms:

Although alpha-amylases are isolated from various microorganisms, fungal and bacterial-derived enzymes have dominated industrial applications (Tanyildizi *et al.,* 2005). The greatest advantages of employing amylase microorganisms are the low volume production costs and the ease of manipulating microbes to create enzymes with acceptable characteristics (Lonsane and Ramesh, 1990). The *Aspergillus* species contain many extracellular enzymes, with amylases being the most important for industry (Hutcheon *et al.,* 2005). Filamentous funguses such as *Aspergillus oryzae* and *Aspergillus niger* contain huge numbers of enzymes, commonly in use in the industry. Because of its ability to produce a variety of proteins and enzymes, including amylase, of significant importance. Filamentous fungus, such as *Aspergillus oryzae* and *Aspergillus niger*, generate vast quantities of enzymes commonly utilized by fermenters. As a suitable host for heterologous protein processing, *A. oryzae* has received a great deal of interest, due to its capacity to produce high-value proteins as well as industrial enzymes like as e-amylase. *A. oryzae* has been utilized in the Orient as a popular drink and fermented meal for more than 2000 years. *A. oryzae* is recognized for heterogeneous protein and enzyme production and for being a commercial source of α-amylase and glucoamylase enzymes (Jin *et al.,* 1998). Few more are given below Table 1.

Table 1. Bacterial and fungi which are source of α-amylase enzyme producer.

Bacteria	Fungi
Bacillius subtilis	*Aspergillus oryzae*
Bacillius licheniformis	*Penicillium fellutanum*
Bacillius cereus	*Thermomyces lanuginosus*
Bacillius amyloliquefaciens	*Aspergillus niger*
Bacillius coagulans	*Penicillium roquefortii*
Chromohalobacter sp.	*Engyodontium album*
Halobacillus sp.	*Penicillium chrysogenum*
Halomonas meridiana	*Penicillium janthinellum*
Rhodothermus marinus	*Pycnoporus sanguineus*

4. Biotechnological applications of Alpha (α)-Amylase in Industries

There are several biotechnological applications of alpha (α)-amylase in several industries and organization all over the world. But some major industries are given below (Figure 2).

4.1. Food Industries:

With the advent of contemporary biotechnology, the food business has altered drastically. There is several research in the food business on the beneficial application of genetic enzymes. Amylase is frequently employed in processed foodstuffs, including baking, fermentation, and digestive aid preparation, cake making, the generation of fruit juice, and starch syrup production (Zeman and McCrea, 1985). Some of the food related uses include corn syrups, maltose syrups, glucose syrups and juices, as well as fermenting and backing alcohol (Mojsov, 2012). These enzymes may be put into bread dough and then fermented by the heifer, breaking up the starch from the flour to tiny dextrin. The adding of alpha amylase in the paste boosts fermentation pace, reduces the viscosity of the paste, enhances the end product's volume and form. It also generates additional sugar in a dough, increasing

the flavor of the bread, the color of the crout and its toasting properties. In addition to the manufacture of fermentable chemicals, -amylase also has an anti-staling impact on bakers and improves the preservation and shelf life of baked goods. Thermo-stable stearothermophilus amylase is currently commercially utilized in the baking business (Ogasahara *et al.,* 1970; Gupta *et al.,* 2003).

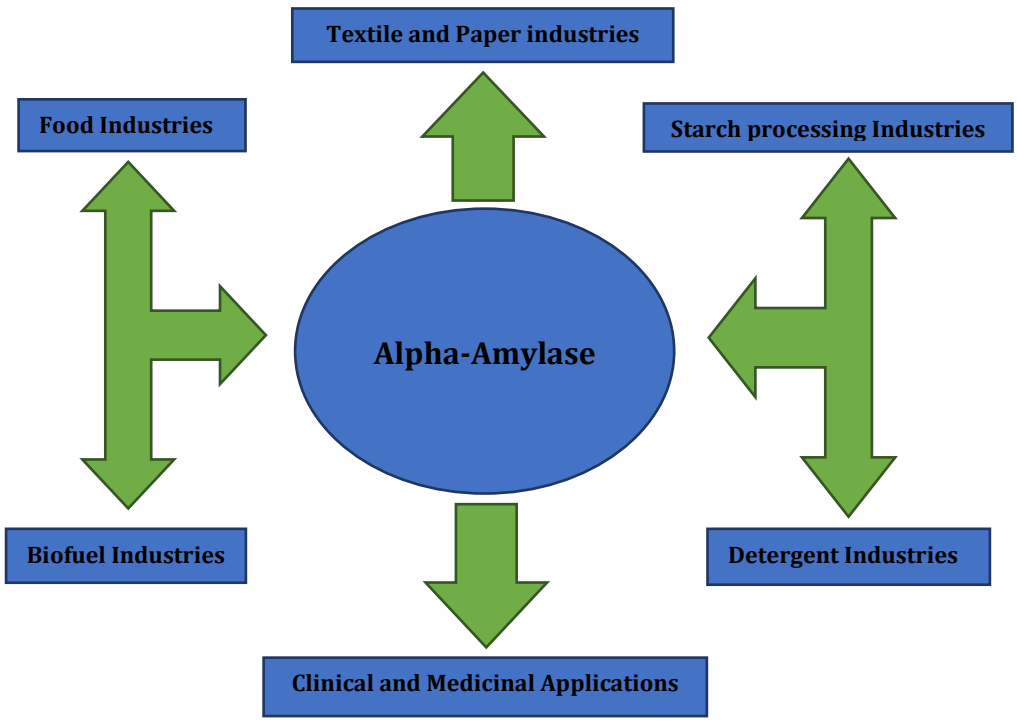

Figure 2. Major biotechnological uses of alpha (α)-amylase

4.2. Starch Processing Industries

The starch industry is the principal marketplace for alpha amylase, utilized for hydrolyzing and transforming starch into fructose and glucose syrups in the liquefaction process. Gelatinization involves the breakdown of starch granules and a viscous solution; partial hydrolysis and lack of viscosity are involved in liquefaction; additional hydrolysis of glucose and maltose is required. This method involves the employment of highly thermostable alpha-amylase, which relies on the environment and may operate at temperatures between 70 and 100°C (Regulapati *et al.,* 2007). The use of alpha-amylases in thermostatics is widespread as it decreases the danger of contamination and response time, which leads to significant energy savings. D-glucose polymerization is also reduced to isomaltoses by hydrolysis at higher temperatures (Maarel *et al.,* 2002). Bacillus amyloliquefaciens alpha-amylase was originally employed, however since then Bacillus stearothermophilus or *Bacillus licheniformis* alpha-amylase have been utilized. It was used. As Bacillus species' enzymes are exceptionally thermostable and have excellent methods of expressions in these enzymes, these enzymes are important to biotechnological operations of large scale (Ogasahara *et al.,* 1970; Prakash and Jaiswal, 2010).

4.3. Biofuel Industries

In recent years, several research initiatives have been undertaken to create microbial strains with enhanced ethanol production capacity and to increase the performance of process ethanol. Moreover, the biotechnological, genetic, and physiological properties of Saccharomyces cerevisiae are more than other microorganism which makes it the appropriate microbial strain for bio-ethanol production. One of the most successful technique of enhancing the efficiency of bioethanol is to isolate yeast mutant cells which are resistant to high concentration of ethanol, and which produces more bioethanol. Recent study has shown that suitable yeast mutant strains have been identified to enhance the bio-ethanol production (Mobini-Dehkordi *et al.,* 2008; 2011). Ethanol is the most often used fluid biofuel. Because of its inexpensive cost and availability across most areas the world starch is the most widely utilized substratum for bioethanol production. Starch must first be solved and then exposed to two enzyme processes in order to create fermentable sugar. The sugar processing of starch utilizing an amylolytic microbe or enzymes such as glucoamylase and -amylase is accompanied by a fermenting process in which the sugar is converted to ethanol by an ethanol-producing microorganism such as yeast (S*accharomyces cerevisiae)* (Moraes *et al.,* 1999; Öner, 2006; Sanchez and Cardona, 2008).

4.4. Detergent Industries

The improved enzyme has exhibited extremely stable surfactants (SDS, Tween 20 and Triton X-100) and good performance with a variety of solid and liquid commercial detergents at 40 °C, which suggests its applications to the detergent sector. Detergent formulations utilize enzymes to increase the capacity of detergent to remove tough stains while making it ecologically friendly. In the production of enzymes, amylases are utilized for virtually 90% of all liquid detergents. The glucoside links hydrolysis of these enzymes may be employed in a range of foods such as spaghets, fruit, cacao, infant food, barbecued sauce, and gravy. They can be used in a wide section of food. In both detergent and dishwasher situations, the treatment of colored stains is of significance. As starch attracts various types of particulate earth, it is important for whiteness to remove starch from surfaces. Hydrolytic enzymes with detergent constituents are stable and compatible, which is determined by how suitable their formulation is to be utilized. In the presence of proteases, it is challenging to utilize amylases in detergent formulations since the enzyme should be stable and have optimal activity in commercially used formulas (Mitidieri *et al.,* 2006; Hmidet *et al.,* 2008).

4.5. Textile and Paper Industries

Amylase of the Bacillus strain is utilized in the clothing business for a long time. In the textile business, it is extremely promising to decorate grey fabrics with raw amylase. The influence of various enzyme levels on alpha amylase degree of grey tissues was examined. The results were examined. The form of the tissue has been seen to increase to some extent when the enzyme concentration increases. However, while the enzyme concentration was higher than the optimum limit, it was harmful to the desizing of the material (Haq *et al.*, 2010). Alpha-amylase is utilized for altering the starch of coated paper in pulp and paper industry to provide low-viscosity, high-molecule starch. The coating process increases the paper's writing efficiency through the adequate smooth, firm surface. The viscosity of natural starch is too high for a paper-size application, but it may be altered by degrading the polymer partially using amylase in batch or continuous processes (Fryer and Asteriadou 2009).

4.6. Clinical and Pharmaceutical Industries

Alpha-amylase may be beneficial in pharmaceutical and fine chemical industries if enzymes with appropriate characteristics can be manufactured. Pharmaceutical researchers were especially interested in biodegradable polymers, both natural and manufactured. Parent rally mediated delivery systems regulate the rate of medication release utilizing biodegradable polymers (Fogarty and Kelly, 1980; Dumoulina *et al.,* 1999).

5. Conclusion:

Amylase enzyme is utilized for different industrial and minor applications such that amylase is employed for decades in stuttered industries, and while the enzyme produces efficiently many microbial sources, only a few strains of fungi and bacteria fulfil the industrial needs. Amylase is one of the most significant industrial enzymes utilized and is increasingly considered helpful for bio-pharmaceutical applications. They are important tools in medical and clinical chemistry. In various sectors, the enzyme has a large range of applications. There are only a few instances of this: food products, textiles, paper brewing, baking, medicinal products, starch processing and detergent. As a consequence of biotechnological development, the number of biotechnological applications in many disciplines, such as pharmaceuticals, medicines and analytical chemistry has increased.

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An Analytical Study of Historical Contribution of Muslims Scientists in the Field of Sciences and its Applications as Practical Knowledge during 680-1000 A.D: A Review

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Abstract

The paper primarily deals with Muslim's contribution in Science. As Islam declared education as a religious obligation and Islam also proved itself as a torch bearer of knowledge of the world after its advent in Arabia and extended its boundaries from Arabia to the edge of Asia and Africa. The Muslim rulers were the great patronage of modern sciences i.e., Physics, Mathematics, Chemistry, Astrology and Medicine. Among the different Muslim Scientists Prince Khalid, Imam Jafer Sadiq, Jabir Bin Hayayn, Abu Ali Seena, Abul Qaim Zaharavi, Abu Rehan Al-Baironi, Muhammad Bin Yahaya Idreesi, Muhammad Bin Zikriya Al-Razi, Ibn-ul-Haitha, Abu Nusa AL-Khuarzami, Ibn-e-Nafees and many other were very important. The Muslim Scientists were the followers of practical knowledge, and they always propagate the application of knowledge as practical rather than oral or theoretical. This paper highlights the role and contribution of Muslim Scientists in the field of Science while it also explores that Muslims were the founder of scientific knowledge in the world.

Keywords: Islam; Knowledge; Science; Education; Obligation; Practical

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1. Introduction:

Muslims always considered the great patronage and custodians of sciences in their entire history and the promoted scientific knowledge. The common perception about the Muslim is not based on reality that Muslims only promoted religious education and traditional knowledge (Hitti, 2002). They have no role in the uplift of modern and scientific knowledge, it seems to be an exaggeration only While Muslims are considered as the founders and architects of science. There is no denying the services and achievements of Muslims in the field of science. Muslims are the inventors of science who not only laid the foundation of science but also nurtured it (Siddiqui, 2008). Be it mathematics or physics, biology or botany and medicine or history, Muslims became educators. But in modern times, their decline and the supremacy of the West have eclipsed their supremacy, which has led to the extinction of their achievements. Today, the proud intellectuals of Europe, who were extremely backward in terms of knowledge in the Middle Ages, have become educators of knowledge and have forgotten the services of Muslims. So, this article is actually a brief introduction to the scientific services of Muslims which not only mirrors the past of Muslims but will be helpful in inspiring guidance of today's students and researchers. Through this research paper, an attempt has been made to highlight the significant deeds of Muslims (Siddiqui, 2008).

1.1. Objectives:

- This paper highlights the role and contribution of Muslim Scientists in the field of Science.
- While it also explores that Muslims were the founder of scientific knowledge in the world.

1. 2. Research Methodology:

The research paper primarily deals with the historical knowledge as a title Muslim contribution in the field of Science therefore historic method of research based on qualitative approach has used with the help of documentary sources i.e., books, articles, essays (Hitti, 2002).

1.3. Review of Literature:

Literature is very important component of research and for this research paper the most relevant has been used including primary and secondary books i.e., History and Philosophy of Science, The Outline of History, The Scientific Outlook, The Making of Humanity, Islam and Evolution of Science, Quranic Concept of History, A Short History of Science, Makers of Chemistry, Introduction to the History of Science and Musilman Sainsdan.

2. Discussion on Topic:

Science is one such knowledge of the system of nature which is derived from observation and experience. The Greeks have a place in human world history in recognizing the importance and usefulness of science by adopting a serious attitude towards science while establishing a formal opinion about science with natural phenomena and facts (Hitti, 2002). The Greeks had no value in science in the ancient time. But it was the Greeks who understood science and began it regularly -The Greeks put the ideas of science first. He took the path of curiosity and reflection but did not attach much importance to experiments. That is why they could not make any significant progress in the experimental sciences, i.e., Physics, chemistry, medicine, biology and botany. They introduced mathematics and geometry to the

sciences. Because there was lacked experience- one of the writers said about ancient and initial stage of science. All this was truly scientific but the bare chronical of fact is only one side of science. There was no tendency to look for reason or to invent unifying theories (Hull, 1959). It is true that Greeks were limited to their region and the thought of Greeks was hampered by a want of knowledge that is almost inconceivable to us today. They had no knowledge of the past mankind at all; they had no knowledge of geography beyond the range of the Mediterranean basin and the frontiers of Persia (Wells, 1920). The Greeks observed the world as poets rather than as men of science said by Russell (1949). Similarly, the scientific method in the development of science, which has a prominent place in modern science and is considered to be the main axis of scientific progress and evolution, was invented by Muslim scientists. Scientific theories existed before the Muslims, but the Muslims introduced the criterion of assumption, observation and experience. Briffault believes that Muslims are part of the experiments in science as he said, what we call science arose in Europe as a result of a new spirit of inquiry, of new methods of investigation, of the method of experiment, observation, measurement, of the development of mathematics in a form unknown to the Greeks. That spirit and those methods were introduced into the European world by the Arabs (Briffault, 1991). Muslim studied particular facts on the basis of experiments and drew inferences in the form of general principles or scientific laws, in other words, the inductive method used to be employed (Saud, 1986).

Allama Iqbal also pointed out as, the first important point to note about the spirit of Muslim culture then is that, for purposes for knowledge, it fixed it gaze on the concrete, the finite. It is further clear that the birth of the method of observation and experiment in Islam was due not to a compromise with Greek thought but to a prolonged intellectual warfare with it. In fact, the influence of the Greek who, as Briffalult (1991) says, were interested chiefly in theory, not in fact, tended rather to obscure the Muslims' vision of the Quran, and for at least two centuries kept the practical Arab temperament from asserting itself and coming to its own (Iqbal, 1977). The science of chemistry has a special place in the scientific sciences. In the field of chemistry, Muslim scientists have worked with such skill and dedication that there is no precedent for it. Until the seventeenth century, Muslims held a degree in chemistry. Muslims founded experimental research. Thanks to which mathematics and chemistry got a special boost. The sciences that Muslims initially introduced or nurtured include zoology, botany, mathematics, chemistry, medicine, physics, and astronomy, and their services are invaluable (Siddiqui, 2008).

2.1. Chemistry

Khalid bin Yazid died in 83 AH was a master of scientific knowledge, especially in Chemistry. Khalid wrote many books (Zubair, 1978) on the knowledge of chemistry. Khalid was a great chemist. He was the first Muslim scientist to write a book on chemistry, which he named Al-Mara *'Al-Badi' Fi Fak Al-Ramz Al-Mani* Khalid also, claimed that he could not become caliph despite being a prince (Saleem, 1981). But he is proud of the fact that his scientific ability is second to none and he has brought chemistry to its peak. The famous historian Ibn Al-Nadeem believes that there was no chemist greater than Khalid at that time and that Khalid wrote more than a dozen books on alchemy (Al-Nadeem, 1986). Some of his few famous books were following.

1- کتاب المرالبدیع فی فک الرمز المنیع 2- کتاب الفردوس 3- کتاب العرارات

4- کتاب الصحیفۃ الکبیر 5- کتاب الصحیفۃ الصغیر 6- کتاب وصیۃ الی ابنہ فی الصنع

Before the advent of Islam in Arabia, there was a tradition to remember the ancestry or family tree and Arabs always felt proud on their memory. While Arabs emphasized on the great importance of the knowledge of genealogy. The Arabs had a historical consciousness, they continued the past by remembering the days of war with genealogy and documentation (Siddiqui, 2008). After Khalid, the

most successful in the field of alchemy is Jabir bin Hayyan, a famous Muslim scientist of the Abbasi era. He had a privileged position in chemistry. It was Jaber who first used not only metals but also successfully converted low-cost metals into precious metals such as gold. He had the honor of discovering many chemical compounds. And these compounds have been successfully used. In which he succeeded in protecting iron from rust and painting glass, for this Jaber made varnishes to dye clothes and protect iron from rust and successfully tested manganese dioxide for the first time to make glass (Singer, 1948). That is why in Arabia, Jabir Ibn Hayyan is called the father of alchemy. He performed many experiments in science. He introduced new rules and ideas for the first time in chemistry. Jaber made new history by developing chemistry on a scientific basis and by developing an experimental basis. He also successfully experimented with blowing the essence of objects through the process of refining (Singer, 1948). The first essential in chemistry is that should perform practical work and conduct experiments, for he who performs not practical work nor makes experiments will never attain to the least degree of mastery. But thou, O my son, do thou experiment so that may acquire knowledge. Scientists delight not in abundance of material; they rejoice only in the excellence of their experimental methods (Holmyard, 1931). Jaber thinks that the most important thing in chemistry is experience, and if you want to get the right knowledge, then get knowledge from experiments. Authenticate knowledge gained in the light of experience by relying on experience, taking practical steps, Jaber set up a laboratory in the light of scientific methods, experiments for chemical processes in it. Jaber set up this laboratory at Kufa in Iraq, a minimalist laboratory with scientific equipment for experiments in chemistry, which has long been the center of scientific activity. The laboratory was built in Kufa with the Damascus Gate. It was here that Jaber performed chemical experiments to produce various acids, as well as a piece of gold from a chemical process that remained in the same laboratory for two years (Holmyard, 1931).

Neutral people in the West believe that Jabir was a complete scientist who was familiar with the process of dissolution, distillation, distillation, and making acid. The most famous alchemist of Islam, Jabir-Ibn-Hayyan, seems to have a good experimental knowledge of a number of chemical facts. "He was acquainted with crystallization, calcination, solution, sublimation, etc., and attempted to explain their nature. He described methods of preparing steel and other metals; dyes for cloth, leather, and hair; varnishes for water-proofing cloth and protecting iron; and substitutes for inks containing gold. He knew the use of manganese dioxide in glassmaking. He was familiar with citric acid and knew how to concentrate acetic acid by distillation of vinegar, and he discovered nitric acid (Sarton, 1976). Abu Bakar Razi was perhaps the first to criticize Aristotle's first figure. It is a mistake to suppose that the experimental method is a European discovery. Europe has been rather slow to recognize the Islamic origin of her scientific method. But full recognition of the fact has at last come (Iqbal, 1977).

2.2. Physics

Like chemistry, Muslims have made unforgettable achievements in the field of physics. Dr. on the experimental path out of the ideological influence of the Greeks. Ibn al-Hathem is credited with experimenting in physics. Ibn al-Hashim provided the basis for this knowledge. He developed the laws of light with regard to physics and wrote a famous book on it called Kitab-ul-Manazir. In it, he has copied his own research based on observation and experiments. This book consists of 1500 pages, and he discussed the rules and laws of physics This book is one his masterpiece (Zubair, 1978). It was generally thought by the Greek, Roman and Muslim scientists that rays are emitted from the eyes towards the objects seen. Plato suggested that there was another set of rays which emanated from the object seen. Ibn-al-Haithum changed the traditional view by putting forward the theory that the objects are seen by the rays passing from them towards the eye and not by the opposite process (Saud, 1986).

His foundation study on the Burning-Spheres represents a real scientific advance and exhibits a profound and accurate conception of the nature of focusing, magnifying and inversion of the image, and of formation of rings and colors by experiments. This work is for beyond anything of its kind produced by the Greeks (Singer, 1948).

2.3. Mathematics

Born in 736, Yaqub Ibn Tariq was the leading mathematician of his time who changed the concept of the Hindu cooker according to which he divided the circle into 96 parts. He wrote several magazines in Arabic on the subject. Three of which were as Appearance Tables, Spherical digits, curves (Siddiqui, 2008). Muhammad Ibn Musa al-Khwarizmi had a long history of mathematics. For the first time, he introduced the equation of algebra. His book Algebra Al-Maqbalah is very famous and his dozens of books in mathematics make him immortal. This book has been read in Europe for centuries-He also prepared tables on trigonometry and astrology. A person who born in Kufa in the ninth century, Yaqub ibn Ishaq al-Kandi was a mathematician. He authored 34 books on mathematics and geometry, including 22 books on physics, and 241 books on the scientific sciences and social sciences. The book Fi Al-Khatwut Al-Zarb and Al-Adal Al-Ashir are famous (Hitti, 2002).

2.4. Biology

Abu Jafar Ahmad Ibn Muhammad, while collecting the trees of Spain and Africa, first suggested their Arabic, African and Latin names. His most famous book is Al-Adwiya Al-Mufrida (Khaldon, 1985). Abdul Malik Asmi was also other important scientist who worked on different animals and his famous books are Kitab-ul-Wahoosh, Kitab-ul-Abil, Kitab-ul-Khail, Kitab-ul-Shat (Siddiqui, 2008).

2.5. Medicine

Muslim played a progressive role in the foundation of Medicine. In Islamic history medicine also popularly called as Tibb-i-Yunani. But before the Muslims medicine had not been properly studied and the material for the early period is scanty. But after it would be useful to refer to available accounts, as the history of medicine is linked up with the development of medieval science and provides a good index of during Muslim contribution (Siddiqui, 2008). The proper, regular, and formal study and foundation of medicine was laid during the Abbasid period. Haroon and his son Mamoon were the great patronage of knowledge and scholars, and they called the various doctors from India were invited to Baghdad. Similarly, in the reconstruction of medicine and the art of surgery, Muslim scientists worked hard and provided the basis for progress. The Arabs did not blindly imitate the Greeks, but acted with great understanding (Saleem, 1996). However, on a more careful study of Arabic manuscripts and of Arabic medical literature, we find that they did contribute to our knowledge of Anatomy in several ways- Farther, the Arabs were not blind followers (Amin, 1946). One of these breathers is named Abu al-Qasim al-Zahrawi, whom Europeans remember as Abulcasis. Al-Zahrawi was the founder of surgery. He is considered to be the inventor of modern surgical science and the greatest medieval surgeon (Hitti, 2002). Sarton paid homage to Al-Zahrawi in these words, Abu-l-Qasim (Abulcasis) was the greatest Muslim surgeon; he exerted a very deep influence upon the development of European surgery down to the Renaissance (Sarton, 1976).

Abu al-Qasim al-Zahrawi's special achievements include the treatment of cataracts, the prevention of bleeding with cold water, and the discovery of anesthesia before surgery. At Bait-ul-Hikmah the important Sanskrit works translated into Arabic was undertaken. After that, the first

detailed reference to the subject is by Barani who gives details prominent physicians of the reign of Balban. Foremost in the latter reign were Maulana Badar-ud-din and Maulana Hamid Mutris, both from Damascus. They were not only practicing physicians but were also good teachers and taught Standard works on medicine to aspiring students (Hitti, 2002). One of the important names among Muslim scientists is Abu Bakar Muhammad Ibn Zakariya al-Razi. About whom Anan was an expert in medicine. Europeans also consider him the ruler of the medical world. The Arabs write about them with pride as one of the historians said about his status that he was not only famous in Arabs also his books were the part of curricula in Europe.

هوا ابو بکر محمد بن زکریا الرازی ولد فی ا العراق سنۃ 420 ھجری واھتم بدراسۃ الطب والصیدلۃ فی کتب الیونان والھنود ولم یکتف بالقراءۃ بل کان یعتمد فی طبہ علی التجربۃ والوصف الشامل لاسباب المرض وعلامتہ وطرق علاجہ وکان یجربہ الادویۃ علی الحیوانات قبل ان یعالج بھا الانسان تبلغ مولفاتہ مائتین واربعۃ عشر کتابا اعتمد علیہا الطب ففی اوربا منذ القرن السابع عشر۔

Al-Razi received a degree in medicine, and his research reached European educational institutions. And Europeans began to use it, relying on his research (Siddiqui, 2008).

2.5.1 Ibn-e-Nafees

Another name in the field of medicine is Muhammad Ibn Ali Ibn Abi al-Hazzam, who lived in Damascus in the early seventh century. He gained access to medicine. During his stay in Cairo, he conducted extensive experiments on the circulatory system. And discussed the cardiovascular system. He wrote more than a thousand books and journals on medicine as Barni said about him;

وقد اکتشف الدورۃ الدمویۃ وتجویف اللقلب والف کتبا کثیرۃ فی الطب کانت مرجعا۔

Bin Nafees also mastered medicine and other sciences, as it is said that he wrote thousands of books on hadith, jurisprudence, and grammar (Hitti, 2002).

2.5.2. Al-Idreesi

Another important name among the eminent Muslim scientists is Muhammad Ibn Abdullah Ibn Idrees, who belonged to the Maghreb Arabs. In addition to geography, the planet, the judges worked on plants, animals and medicine, and his famous book on this is Nazhat al-Mushtaq which is being taught in Europe and in Ilam-ul-Ulama it said about this book - This is a book full of medical virtues. Never before has such a detailed book been written on the subject of medicine (Siddiqui, 2008). Even in Subcontinent Muslim Physicians did not ignore the study and work on medicine and they continue it during the entire sultanate period. Firuz Shah promoted medical works composed in his reign was Tibb-t Firuz named after him. Rahat al-Insan, a work on popular medicine composed 778/1376, was also dedicated to him (Siddiqui, 2008). Muslim scientists worked on medicine and its sources practically from the beginning, and there is an important link in this chain. The important medieval work of this type was Madan al-Shifa-i Sikandar Shahi, also known as Tibb-i Sikandari. This was completed in 919 AH during the reign of Sikandar Lodi, by his court physician, Mian Bhowa. The brief account given above is enough to show that the study practice of medicine was not neglected during the early Muslim era and other spheres, material available in past as well as classical Islamic was utilized (Hitti, 2002).

3. Conclusion

There is no doubt that after the Greeks, Muslims were the most prominent nation who played the most splendid role in the dispensation of scientific knowledge all over the world without any partiality and discrimination. They started practical knowledge and set laboratories in different areas

as compared to Greeks who emphasized on theoretical knowledge. If we look at the development of Europe and the West in the field of science today, remember it carefully that these are the effects that were observed by the people of the West in Spain in the Middle Ages, and then known as Andalus. It was the research and experience of the Muslims that the West borrowed and attributed to them their achievements, forgetting the deeds of the Muslims. While denying the knowledge of Muslims by classifying them as ignorant people of the third world. Orientalists in particular used misleading propaganda against Muslims. If we talk about Islam and science, it is as clear as day that Islam has made the acquisition of knowledge a religious duty and has emphasized research. The teachings of Islam were rationally developed by Muslim scientists and in a short time they achieved perfection in the scientific sciences. Under the patronage of the rulers in the Islamic Empire, an atmosphere of science and research was established in which people from different countries came to Baghdad and Cordoba to acquire knowledge. He began researching scientific theories through practical experiments. The Arabs enlightened the world by providing new foundations for science. He changed the nature of knowledge and placed it in front of new generations. These sciences included chemistry, physics, medicine, mathematics, astronomy, history, geography, geology, biology. The purpose was not only to reform the Greeks in all fields of science and art, but also to work for the development of the sciences. Similarly, it is clear that research and experiments were formally initiated by the Arabs, which was previously limited to observations. By becoming a pioneer of research based on experiments with Arab observations, not only did they take the lead in the world, but the process of making laws from observations and experiments also created confidence in parliamentarians. Similarly, Muslims continued to pursue science and research. And for a long time, he was known as the master and educator of non-scientific sciences and his research became the first step in the development of the European education system. And from here the West was introduced to the scientific endeavors of Muslims. And he became aware of the works of scholars such as Jabir Ibn Hayyan, Bu Ali Seena, Ibn al-Hashim, Abu al-Qasim al-Zahrawi, Abu Bakar al-Razi, Idreesi, al-Khwarizmi, al-Biruni and Ibn Khaldon. So, in contrast to the prejudiced attitude, the Orientalists and the West have to accept that the progress of science cannot be considered the success of any other nation without involving the struggle of the Muslims. Without Muslims, science and research will remain an incomplete chapter in history. This research paper will be helpful for the students not only but for the researchers who are interested the topic Islam and Science.

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