

## Influence of Edapho-Physico-Chemical Properties along altitude and aspects on the density of Medicinal Orchids *Habenaria intermedia* D.Don. and *Microstylis wallichii* Lindl. in India

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**Abstract:** Orchids are jewels of plant kingdom depicting aesthetic and usufructs value for noble purpose. These are also used globally for medicinal purpose specially in Chinese, Tibetan, European and Indian system of medicine besides their bioactive compounds used in modern system of medicine. In Ayurvedic system orchids like *Habenaria intermedia* and *Microstylis wallichii* are placed in Asthavarga, Madhuraverga, Jeevaniyaverga and Kakoli group. The physico-chemical edaphic attributes associated with these orchids along different altitude and aspect provide information for *in situ* and *ex situ* conservation of orchid species. *H. intermedia* require proper aeration and less moisture around their roots while *M. wallichii* prefer damp cool habitat with high moisture content but require good aeration as the species has superficial root system spread on litter layer in Himalayan region. Increase in nitrogen, potassium and organic carbon content with decrease in altitude also limits the density of *H. intermedia* while phosphorus with increase in altitude increases its density. Nitrogen and organic carbon was low in southwest aspect compared to other aspect and also support the relatively high density of *H. intermedia* showing preference for low nitrogen, potassium and organic carbon and high phosphorus. In case of *M. wallichii* the high density of species was observed with respect to high nitrogen and organic carbon. The density of *H. intermedia* was recorded (6.4 plants/ m<sup>2</sup>) at NW at 2400 m altitude, 7.3 plants/ m<sup>2</sup> in SW at 2200 m altitude and 4.9 plants/ m<sup>2</sup> in SW aspect at 2000 m altitude. Similarly in *M. wallichii* at 1800 m altitude recorded density of 33.50 plants/ m<sup>2</sup> in NW aspect with IVI 94.34 while density 16.30 plants/ m<sup>2</sup> was found in NW aspect at 2000 m altitude and 40.19 plants/ m<sup>2</sup> at 2200 m altitude in SW aspect. The present paper discusses the interrelationship of these orchids with soil attributes along altitude and aspect in Himalayan region.

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### 1. Introduction

Orchids were first found between 370-285 B.C. while searching for medicinally important herbs. Now they are becoming an increasing source of attraction and fascination not only to plants taxonomists and conservationists, but also to Ayurveda specialist, as many orchid species are regarded as natural herbs of high medicinal values. A mosaic of orchid flora is found in Himalayas, which is richest region of India in terms of number of species and genera of orchids. In India out of 1,300 orchid species, 876 species are in Himalayas of which 240 species inhabited in Uttarakhand hills (Deva and Naithani, 1986; Pangaty *et al.*, 1992).

Orchids have been used as medicines in all parts of the world. These are used in Chinese, Tibetan, European and Indian system of medicine as well as some of the bioactive compounds used in modern system of medicine. Lawler (1978) reviewed the medicinal orchids of world in detail. In Ayurvedic system of medicine some orchids like *Habenaria*

*intermedia*, *H. edgeworthii*, *Microstylis wallichii* and *M. muscifera* are placed in Asthavarga, Madhuraverga, Jeevaniyaverga and Kakoli group (Achyra Balkrishna *et al.*, 2012, Chuhan *et al.*, 2007, Dobriyal *et al.*, 2003).

India as well as Himalayan region specifically is expected to loose most of the valuable orchid wealth that are of medicinal, horticultural and aesthetic values (Rao, 1979; Arora and Mukherjee, 1983 and Handa, 1986). Like many other medicinal plants of Himalayan region *H. intermedia* and *M. wallichii* are also facing extinction in its natural habitats due to increased market demand and consequently excessive destructive harvesting. Further, development works in hill areas like, big dam projects, roads and expansion of agricultural lands deteriorating the very survival of these species. The inherent vulnerability and physiographic instability of mountain ecosystems created the Himalayan region an ecologically fragile biogeographic zone in India. Further, the demographic, economic and social changes have

important consequences on conservation of its valuable flora.

*Habenaria* is a large genus of orchids found in temperate and tropical regions of the world. *Habenaria intermedia* D. Don. commonly known as Ridhi, is an undivided or lobed tuberous rooted, monopodial growth, terrestrial orchid recorded in Garhwal Himalayas at 1,500 to 2,800 meter elevation. Its stem terete, 25-50 cm long, bears 4-5 long, acuminate leaves with round base scattered to 10 cm long. The inflorescence is fairly long with many small to large fibers. The plants flower during rainy season July-August (Anon, 1998, Dobriyal, 2002). The tuberous root of *H. intermedia* are used in Chyvanparash, as an important ingredient of Ashtawarga group of formulations and can be used as tonic, blood purifier, rejuvenator and life span promoter. *Microstylis* is a genus of about 200 species of mainly terrestrial and rarely epiphytic orchids, often with pseudobulbs. *Microstylis wallichii* Lindl. Syn, *Malaxis acuminata* D. Don is a terrestrial orchid with sympodial growth found in temperate and subtropical regions of Himalayas between 1800 to 2300 meter elevations. The plant flower during July - August (Anon, 1998, Dobriyal, 2002a). The pseudobulb is an ingredient of Ashtawarga, used in preparation of Chyvanparash, enhances sperm formation and is also known to cure tuberculosis (Gupta, A., 2016).

Considering the fact that the natural population of orchids has reduced to critically low level, there is urgent need to conserve these important medicinal orchid species in wild. Therefore in order to formulate an *in-situ* conservation strategy for *H. intermedia* and *M. wallichii*, resource availability in different altitudes and aspect of occurrence are extremely essential, which would help to develop an ecological understanding of the species behaviour in nature and its relationship with physico-chemical properties of soil. This would also guide in identifying the factors, which govern its distribution, growth, density and regeneration in the natural habitats. Since the population of *H. intermedia* and *M. wallichii* is fast depleting in its natural habitats and their natural reservoirs are likely to exhaust soon at the current extinction rate. It becomes essential to think of alternative sources for the continuous availability of the raw material to the practitioners of Ayurveda and other indigenous systems of medicine besides ensuring a sustained supply of quality raw material to the pharmaceutical industry. The viable option therefore is to undertake cultivation of the species in non-forest areas in the region. Thus in order to preserve these rare, endangered and endemic medicinal plant wealth for future generation there is an urgent need for development of concrete

conservation plans based on indigenous knowledge with scientific inputs for conservation, management and sustainable development of the species (Dobriyal et al, 2002, Chaudhari & Dobriyal, 2002). For conservation (*in situ* and *ex situ*) of any species, a sound knowledge of environmental factors and ecology are prerequisites. Therefore, in order to formulate strategies for conservation and propagation of the species, present study deals with quantitative information on physiochemical properties of soil associated with natural habitat of *H. intermedia* and *M. wallichii*.

## 2. Materials and Methods

The study have been undertaken in the area of Chakrata sub division of Garhwal Himalayas in Uttarakhand, India between 31°26' - 31°02' N latitude and 77°38' - 77°4' E longitude at an altitude of 1,800 to 2,400 meters above a.s.l. The climate of the area is moist temperate receiving moderate to heavy snowfall. Mean annual precipitation is quite high (8-10 %), is in the form of snow during winter (December to February) and about 65% as rain during monsoon (June to September). Annual temperature ranges between 20°-25° C and mean annual rainfall is 1,300 mm. The relief is hilly with slope gradient normally ranging 35% to 55%. Main locations covered in Chakrata region were Thanadanda, Lurli forest, Chakrata central, Joshi tibba, Darnadhar, Chaurani, and Nagaukandi. All these sites were in the Chakrata region at a distance of 15-20 km. away from each other. The study sites were selected through stratified random sampling by dividing the whole Chakrata area into five altitudinal zones from 1800 to 2400 meters and in each altitudinal strata three aspects were chosen viz, NW, NE, and SW with reference to species availability. The forest area falls under group '12' (Himalayan moist temperate forest) and sub-group 12C<sub>1</sub> and 12C<sub>2</sub> (Lower and upper Himalayan moist temperate forests respectively) (Champion & Seth 1968). The soils of the area are fine textured, acidic in reaction, non- calcareous in nature and rich in organic matter.

Soil samples were collected from all the fourteen locations and experimental site. Samples were collected randomly from each location at two depths i.e. 0-15 cm, and 15-30 cm to study the physical and chemical properties of the soil. For the texture studies the soil sample was fractionated in two portions viz., more than 2 mm and less than 2 mm size using a stainless steel sieve with appropriate mesh size. Soil with less than 2 mm particle size was used for texture analysis by Hydrometer method (Black, 1965) and percentage of different textural fraction i.e. sand, silt and clay was then calculated. For determination of soil moisture percentage, fresh weight of soil was

taken immediately in the field and then soil samples were brought in laboratory in airtight moisture boxes. The soil was dried in an oven at  $100^{\circ}\text{C} \pm 5^{\circ}\text{C}$  until a constant weight was obtained and soil moisture percentage was then calculated (Wilde *et. al.*, 1985). The organic carbon was determined by Walkley and Black rapid titration method (Jackson, 1967), Total nitroge was estimated by Micro Kjeldahl Method (Jackson, 1967), total phosphorous and total potassium was determined using standard method as described by Jackson (1967).

### 3. Results and Discussion

Soil physico-chemical properties strongly influence the character of vegetation. The nature of substratum (soil profile) is an important factor affecting the vegetation in a given area. Soil texture data presented in Table 1 and 2 Shows that the soil associated with the 2400 m and 2200 m altitude in south west aspect was found to be clay loam in nature while 2000m reflect Silty clay loam. With increasing depth of the soil the percentage of clay and silt was found to be increasing whereas sand was decreasing. In north east aspect with increase in depth sand and silt percent increased but clay percent decreased while in north west aspect in silty clay loam soil, there was increase in sand per cent with increase in depth but no variation in silt and clay percent. When comparing the mean values of clay, silt and sand percentages between different aspects, the highest percentage of clay (33.23%) was found in south west aspect followed by north east (31.25%) and north west (28.38%). The maximum percentage of silt (46.88%) in North West aspect followed by North east (42.75%) and North West (41.13%).

The soil associated with 2200 m altitude in south west aspect was found to be clay loam and sand - clay content decreased with depth while silt content increased. In north east aspect the soil was silty clay loam, sand and silt content increased with depth while clay content decreased. North West aspect also has silty clay loam soil. There was a decrease in sand content and increase in clay content with depth. It was observed that the highest percentage of clay (33.08%) was to be found in North West aspect followed by north east (32.65%) and south west (31.18%). The maximum percentage of silt (46.50%) in north east and north west aspect (46.13%), which are at par followed by south west (42.80%). Sand percentage was maximum (26.03%) in SW aspect. In NW and NE, sand percentage was 20.80 % and 20.85% respectively (Table 1 & 2).

Similarly soil associated with 2000 m altitude in south west aspect was silty clay loam soil showed decrease in sand, silt and clay per cent with increase in depth. In north east aspect soil was again silty clay

loam. The soil showed increase in sand per cent and decrease in silt content with increase in depth. In North West aspect the soil which was clay loam in nature reflected an increase in sand and silt content with increase in depth. It was observed that the highest percentage of clay (34.88%) was found in north east aspect followed by south west (33.15%) and north west (29.33%). The maximum percentage of silt (48.35%) in south west followed by north west aspect (45.00%) and south west (44.93%), which were at par. Sand percentage was maximum (25.68%) in North West aspect which was at par with south west aspect (24.95%) followed by north east (20.20%) (Table 1 and 2).

In *Microstylis wallichii* inhabited areas, the soil properties in 2200 m altitude in south west showed decrease in sand and silt and increase in clay content, while in north east aspect the soil was silty clay loam and there was increase in sand and decrease in silt and clay content with increase in depth. North west aspect also having silty clay loam and sand content decreased and clay content increased with depth. The highest percentage of clay (34.78%) was found in north east aspect followed by south west (33.43%) and north west (33.08%). The maximum percentage of silt (47.68%) was recorded in north east aspect followed by north west aspect (46.13%) and south west (44.43%). Sand percentage was recorded maximum (21.78%) in south west aspect followed by north west aspect (20.80%) and north east (17.68%) (Table 1 and 2). The soil associated with 1800 m in south west aspect was clay loam and has shown an increase in sand and silt content and decrease in clay content while in north east aspect it was silty clay loam soil in which sand content increased and silt and clay content decreased with respect to increase depth. In North West aspect sand content increased and clay content decreased with increase in depth. The highest percentage of clay (34.15%) was found in North West aspect followed by north east (33.45%) and south west (27.48%) which were at par. The maximum percentage of silt (48.63%) was found in North East and North West aspect followed by south west (45.13%). Sand percentage was maximum (27.50%) in southwest aspect followed by north east aspect and North West as 17.93 % and 17.48% respectively. The moisture percent associated with different altitudes and aspects increase with respect to decrease in altitude and increase in depth. North west aspect had high moisture content followed by north east and south west. Comparing data of different altitudes, maximum moisture content (40.08 %) was to be found in 1800 m followed by 2000 m (36.17%), 2200 m (33.67 %) and minimum 32.50% at 2400 m altitude. Maximum moisture (51.25%) was found in north west

aspect at 1800 m and minimum in south west aspect at 2400 m (Table 2).

Soil chemical analysis was also undertaken to ascertain the soil type preferences and nutritional status of *H. intermedia* and *M. wallichii* in different altitude and aspects. Results of studies are indicative to be used to manipulate nutritional requirements that may be required while attempting to cultivate these species for commercial purposes. Analysis of data of various chemical parameters of soil viz., pH, organic carbon, total nitrogen, total phosphorus and total potassium was performed to determine the individual influence of each parameter in relation to altitude, aspect and depth of soil (Table 3). The results indicate that the pH of upper stratum of soil was more acidic than lower stratum irrespective of altitude and aspect. At 2400 m and 2200 m altitude in north west and south west aspect was more acidic than north east aspect. At 2000m there was no significant variation in pH among the aspects while at 1800 m southwest aspects was found with neutral properties. While comparing data of different altitudes, it was noticed that as the altitude increases soil acidity also increased. The interaction of altitude, aspect and depth was found non significant. Within each altitude and aspect, the organic carbon tends to decrease significantly, with the increase in depth of the soil. Irrespective of altitude North West aspect showed maximum organic carbon content (5.54% and 6.08 %). Comparing different altitude and aspect maximum organic carbon (6.08%) recorded in North West aspect at 1800m altitude. The data indicates that the nitrogen per cent in upper stratum of soil was higher than lower stratum. In respective of altitude nitrogen percent was found higher in North West and north east aspect while along the altitude it decreased as the altitude increased (Table 3 & 7). The maximum (0.582 %) nitrogen percent was recorded in North West aspect at 2000 m altitude (Table 3 & 4). The total phosphorus percent in upper stratum of soil was higher than lower stratum. Irrespective of altitude phosphorus percent was found higher in north east aspect while along the altitude it showed no regular pattern. The maximum (0.0590 %) phosphorus percent recorded in northwest aspect at 1800 m altitude (Table 3 & 5). The total potassium percent in upper stratum of soil was higher than lower stratum. Potassium percent with respect to altitude and aspect did not show any pattern. The maximum (1.65 %) potassium percent recorded in north west and north east aspect at 2000 m altitude (Table 3 & 6). There was significant difference in nitrogen, phosphorus and potassium content along the altitude, aspect and depth but interaction of among all these factors was found non significant.

The density of *Habenaria intermedia* was found (6.4 plants/ m<sup>2</sup>) at NW at 2400 m altitude but IVI

highest 17.83 in NE aspect. At 2200 m altitude 7.3 plants/ m<sup>2</sup> and 4.9 plants/ m<sup>2</sup> at 2000 m altitude, which indicated that with increase in altitude there was marked increase in population of the *H. intermedia*. Similarly in *Microstylis wallichii* at 1800 m altitude recorded density of 33.50 plants/ m<sup>2</sup> in NW aspect with IVI 94.34. The density of 16.30 plants/ m<sup>2</sup> was found in NW aspect at 2000 m altitude and 40.19 plants/ m<sup>2</sup> at 2200 m altitude in SW aspect (Table 8). The general observation was *H. intermedia* prefer the open grasslands and soil exposed slopes while *M. wallichii* prefers the shady places under Oak and Oak mixed forests.

Presently, the soils of study area were found to range between loam to silty clay loam to sandy loam in texture but their moisture content, nutrient contents and pH varied with the locality. The sandy and sandy loam soils are better aerated and have poor water holding capacity as compared to loamy soils (Buckman and Brady, 1971). The herbaceous plants are known to prefer rich organic matter, higher amount of available nutrients, moisture, temperature (10-20<sup>0</sup> C) and high light intensity (Collins *et al.*, 1985). Chakarvarty and Chakarvarty (1980) observed a decrease in clay content increases with the organic matter and in the proportion of easily extractable humus with increasing altitude. *Habenaria intermedia* requires proper aeration and less moisture around their roots. *Microstylis wallichii* prefers damp cool habitat with high moisture content but require good aeration as the species has superficial root system spread on litter layer (Manmohan *et al.*, 2011 and 2012). Yadav (1963) has studied soil under the pure plantation of deodar (*Cedrus deodara*) in Chakrata forest reported the highest proportion of coarse sand and lowest amount of silt through the depth. In general, the texture of the soils varies from sandy loam to clay and does not manifest any consistent trend with the depth. Soils are richly supplied with nitrogen and organic matter in the upper horizons and moderate to insufficient quantities in the lower horizons. The content of available phosphorous is low in all the profiles and is more or less uniformly distributed throughout the depth.

Synthesis of data from table 3, 4, 5, 6 and 7 reveals that increase in nitrogen, potassium and organic carbon content with decrease in altitude also limits the density of *H. intermedia* while phosphorus with increase in altitude increase the density. Nitrogen and organic carbon was low in south west aspect compared to other aspect and also support the relatively high density of *H. intermedia*. This shows that the *H. intermedia* prefers low nitrogen, potassium and organic carbon and high phosphorus. In case of *M. wallichii* the high density of species was observed with respect to high nitrogen and organic carbon. In



temperate forest of Shimla the carbon contents were found to vary between 2 to 7 % in the orchid habitats (Vij *et al.*, 1992). Nitrogen rich substratum has often been considered conducive to orchid growth (Sheehan, 1961). *In vitro* studies suggest that requirement for either ammonium or nitrate as a source of nitrogen varies with the species (Vij *et al.*, 1998). Vij *et al.* (1998a) observed in Shimla hills that *H.intermedia* and *Malaxis acuminata* species thrives in varied type of soil in nitrogen content seems to be related to their wide ecological amplitude.

There are several findings, which support that there is change in soil character with the different gradient of altitude. The elevation from 1400 m to 3615 m right from tropical to temperate forest to alpine region of the Eastern Himalayas were analyzed for soil characteristics induced by vegetation along with variation in altitude. All the soil studied was derived from Darjiling gneiss and schist's, consisting of sand stone and quartzites; the soil differences are mainly due to variation in climate conditions and vegetation covers. Organic carbon, total N, humus carbon, humic acid carbon and fulvic acid carbon contents of the soils where significantly and positively corrected (at 1% level) with altitudes (Gangopadhaya *et al.*, 1990). A study of morphology and physico-chemical analysis of temperate soil profiles at high altitudes of North East Himalayas have indicated that the predominant processes of illuviation of bases from upper to lower strata. Surface soil accumulated up to

3.88 % of organic carbon and 0.42% of total nitrogen (Dimri, 1992).

Mycorrhizal association is an integral part of orchid life cycle (Knudsen, 1922) and it is governed by the nutrient status of the *Oreorchis* substratum (Withner, 1974). Since the fungal partner, in such associations, is believed to augment the carbohydrate transport, carbon content of the substratum is an important aspect of orchid nutrition. However mycorrhizal studies were not undertaken in present study. Woodward and Pigott (1975) reported the differences in growth of *Sedum telephium* at different altitudes are probably due small changes in the air, temperature and irradiant. It has been reported by a number of workers that the productivity in terms of total dry weight plants decreases with increasing altitude. There is considerable variation in above ground plant biomass of alpine plant species through the different months. It increased rapidly from May to peak value in September. In October, biomass declined rapidly to a minimum due to death of plants by the advance of winter season (Joshi *et al.*, 1988).

The upper horizons of the soils under silver fir and spruce have higher contents of organic matter, nitrogen etc as compared to lower layers. The compositions of needles, forest litter and raw humus of silver fir and spruce forest at Narkanda H.P. have been estimated by Dhir (1967, 1970). These findings are in consonance with other reported literature in soil characteristics of Himalayan region in different type of vegetation.

**Table 1: Soil texture at different altitude and aspect in relation with orchids *Habenaria intermedia* and *Microstylis wallichii***

Site	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Textural Class
<b><i>Habenaria intermedia</i>, Altitude: 2400 m</b>					
SW	0-15	30.70 ± 0.30	36.75 ± 0.25	32.55 ± 0.05	Clay loam
	15-30	20.60 ± 0.40	45.50 ± 0.25	33.90 ± 0.10	Clay loam
NE	0-15	23.40 ± 0.60	41.50 ± 1.00	35.10 ± 1.60	Clay loam
	15-30	28.60 ± 0.60	44.00 ± 1.00	27.40 ± 0.40	Clay loam
NW	0-15	22.60 ± 0.60	47.75 ± 0.25	29.65 ± 0.85	Silty Clay Loam
	15-30	26.90 ± 0.60	46.00 ± 1.00	27.10 ± 1.60	Silty Clay Loam
<b><i>Habenaria intermedia</i>, Altitude: 2200 m</b>					
SW	0-15	31.30 ± 1.10	36.00 ± 1.00	32.70 ± 0.10	Clay loam
	15-30	20.75 ± 0.25	49.60 ± 0.40	29.65 ± 0.15	Silty Clay Loam
NE	0-15	18.60 ± 0.40	45.50 ± 0.50	35.90 ± 0.90	Silty Clay Loam
	15-30	23.10 ± 1.10	47.50 ± 0.50	29.40 ± 0.60	Clay loam
NW	0-15	23.00 ± 1.00	46.75 ± 0.25	30.25 ± 0.75	Silty Clay Loam
	15-30	18.60 ± 0.40	45.50 ± 0.50	35.90 ± 0.90	Silty Clay Loam
<b><i>Microstylis wallichii</i>, Altitude: 2200 m</b>					
SW	0-15	26.80 ± 1.20	46.10 ± 0.90	28.00 ± 1.20	Silt Clay Loam
	15-30	16.75 ± 1.25	42.75 ± 0.25	38.85 ± 0.65	Clay loam
NE	0-15	14.10 ± 0.90	48.60 ± 0.40	37.30 ± 1.30	Silty Clay Loam
	15-30	21.25 ± 0.75	46.75 ± 0.25	32.25 ± 0.25	Silty Clay Loam
NW	0-15	23.00 ± 1.00	46.75 ± 0.25	30.25 ± 0.75	Silty Clay Loam

Site	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Textural Class
	15-30	18.60 $\pm$ 0.40	45.50 $\pm$ 0.50	35.90 $\pm$ 0.90	Silty Clay Loam
<b><i>Habenaria intermedia</i> &amp; <i>Microstylis wallichii</i>- 2000 m</b>					
SW	0-15	26.80 $\pm$ 0.25	49.75 $\pm$ 0.25	36.35 $\pm$ 1.15	Silty Clay Loam
	15-30	23.10 $\pm$ 0.90	46.95 $\pm$ 0.55	29.95 $\pm$ 1.45	Silty Clay Loam
NE	0-15	15.60 $\pm$ 0.60	49.25 $\pm$ 0.75	35.15 $\pm$ 1.35	Silty Clay Loam
	15-30	24.80 $\pm$ 0.80	40.60 $\pm$ 0.40	34.60 $\pm$ 1.20	Clay loam
NW	0-15	20.60 $\pm$ 0.40	43.50 $\pm$ 1.50	35.90 $\pm$ 1.90	Clay loam
	15-30	30.75 $\pm$ 0.75	46.50 $\pm$ 1.50	22.75 $\pm$ 0.75	Silty Clay Loam
<b><i>Microstylis wallichii</i>, Altitude: 1800 m</b>					
SW	0-15	20.80 $\pm$ 0.20	44.50 $\pm$ 0.50	34.90 $\pm$ 0.10	Clay loam
	15-30	34.20 $\pm$ 0.08	45.75 $\pm$ 0.25	20.05 $\pm$ 1.05	Loam
NE	0-15	11.25 $\pm$ 0.75	49.50 $\pm$ 0.50	39.25 $\pm$ 0.25	Silty Clay Loam
	15-30	24.60 $\pm$ 0.60	47.75 $\pm$ 0.75	27.65 $\pm$ 0.15	Silty Clay Loam
NW	0-15	13.85 $\pm$ 0.35	49.25 $\pm$ 1.25	37.40 $\pm$ 1.40	Silty Clay Loam
	15-30	21.10 $\pm$ 0.90	48.00 $\pm$ 1.00	30.90 $\pm$ 0.10	Silty Clay Loam

\* Mean value of 3 samples, Mean  $\pm$  S.E.

NE- North East aspect, SW- South West aspect, NW- North West aspect

**Table 2 Soil physical attributes at different altitudinal zone and aspect in relation with orchids *Habenaria intermedia* and *Microstylis wallichii***

Site			Sand (%)			Silt (%)			Clay (%)			Moisture (%)		
			Depth (cm)			Depth (cm)			Depth (cm)			Depth (cm)		
Species	Altitude	Aspect	0-15	15-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean
<i>H. intermedia</i>	2400 m	SW	30.70	20.60	25.65	36.75	45.50	41.13	32.55	33.90	33.23	30.50	25.50	28.00
		NE	23.40	28.60	26.00	41.50	44.00	42.75	35.10	27.40	31.25	35.00	29.00	32.00
		NW	22.60	29.60	26.10	47.75	46.00	46.88	29.65	27.10	28.38	45.00	30.00	37.50
	Mean		25.57	26.27	25.92	42.00	45.17	43.58	32.43	29.47	30.95	36.83	28.17	32.50
<i>H. intermedia</i>	2200 m	SW	31.30	20.75	26.03	36.00	49.60	42.80	32.70	29.65	31.18	31.00	27.00	29.00
		NE	18.60	23.10	20.85	45.50	47.50	46.50	35.90	29.40	32.65	32.00	28.50	30.25
		NW	23.00	18.60	20.80	46.75	45.50	46.13	30.25	35.90	33.08	35.00	30.50	32.75
	Mean		24.30	20.82	22.56	42.75	47.53	45.14	32.95	31.65	32.30	32.67	28.67	30.67
<i>M. wallichii</i>	2200 m	SW	26.80	16.75	21.78	46.10	42.75	44.43	28.00	38.85	33.43	38.50	32.50	35.50
		NE	14.10	21.25	17.68	48.60	46.75	47.68	37.30	32.25	34.78	35.00	30.50	32.75
		NW	23.00	18.60	20.80	46.75	45.50	46.13	30.25	35.90	33.08	35.00	30.50	32.75
	Mean		21.30	18.87	20.08	47.15	45.00	46.08	31.85	35.67	33.76	36.17	31.17	33.67
<i>H. intermedia</i> & <i>M. wallichii</i>	2000 m	SW	26.80	23.10	24.95	49.75	46.95	48.35	36.35	29.95	33.15	35.50	32.50	34.00
		NE	15.60	24.80	20.20	49.25	40.60	44.93	35.15	34.60	34.88	38.50	33.50	36.00
		NW	20.60	30.75	25.68	43.50	46.50	45.00	35.90	22.75	29.33	40.50	36.50	38.50
	Mean		21.00	26.22	23.61	47.50	44.68	46.09	35.80	29.10	32.45	38.17	34.17	36.17
<i>M. wallichii</i>	1800 m	SW	20.80	34.20	27.50	44.50	45.75	45.13	34.90	20.05	27.48	33.00	29.00	31.00
		NE	11.25	24.60	17.93	49.50	47.75	48.63	39.25	27.65	33.45	40.50	35.50	38.00
		NW	13.85	21.10	17.48	49.25	48.00	48.63	37.40	30.90	34.15	53.50	49.00	51.25
	Mean		15.30	26.63	20.97	47.75	47.17	47.46	37.18	26.20	31.69	42.33	37.83	40.08

NE- North East aspect, SW- South West aspect, NW- North West aspect

**Table 3 Soil Chemical attributes at different altitudinal zone and aspect in relation with orchids *Habenaria intermedia* and *Microstylis wallichii***

Site			Available Nitrogen (%)			Available Phosphorus (%)			Available Potassium (%)			Organic carbon (%)			pH		
			Depth (cm)			Depth (cm)			Depth (cm)			Depth (cm)			Depth (cm)		
Species	Altitude	Aspect	0-15	15-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean	0-15	15-30	Mean
<i>H. intermedia</i>	2400 m	SW	0.233	0.170	0.202	0.0455	0.0305	0.0380	1.47	1.35	1.41	4.00	2.95	3.48	5.25	5.54	5.40
		NE	0.395	0.310	0.353	0.0465	0.0370	0.0418	1.41	1.36	1.39	3.29	3.07	3.18	6.24	6.55	6.40
		NW	0.370	0.300	0.335	0.0497	0.0326	0.0412	1.30	1.23	1.27	5.95	5.13	5.54	5.73	5.92	5.83
	Mean		0.333	0.260	0.296	0.0472	0.0334	0.0403	1.39	1.31	1.35	4.41	3.72	4.07	5.74	6.00	5.87
<i>H. intermedia</i>	2200 m	SW	0.366	0.285	0.326	0.0365	0.0275	0.0320	1.49	1.40	1.45	3.19	3.00	3.10	5.89	5.70	5.80
		NE	0.386	0.280	0.333	0.0577	0.0451	0.0514	1.57	1.43	1.50	4.60	4.13	4.37	6.07	6.44	6.26
		NW	0.458	0.348	0.403	0.0278	0.0151	0.0215	1.49	1.17	1.33	4.43	3.76	4.10	5.29	5.65	5.47
	Mean		0.403	0.304	0.354	0.0407	0.0292	0.0350	1.52	1.33	1.43	4.07	3.63	3.85	5.75	5.93	5.84
<i>M. wallichii</i>	2200 m	SW	0.454	0.305	0.380	0.0486	0.0308	0.0397	1.17	1.51	1.34	4.39	3.34	3.87	5.81	6.17	5.99
		NE	0.506	0.318	0.412	0.0505	0.0440	0.0473	1.30	1.45	1.38	5.94	4.44	5.19	5.85	6.21	6.03

		NW	0.458	0.348	0.403	0.0278	0.0151	0.0215	1.49	1.17	1.33	4.43	3.76	4.10	5.29	5.65	5.47
Mean			0.473	0.324	0.398	0.0423	0.0300	0.0361	1.32	1.38	1.35	4.92	3.85	4.38	5.65	6.01	5.83
<i>H. intermedia</i> & <i>M. wallichii</i>	2000 m	SW	0.516	0.431	0.474	0.0540	0.0403	0.0472	1.79	1.43	1.61	5.31	4.52	4.92	6.31	6.61	6.46
		NE	0.460	0.330	0.395	0.0481	0.0604	0.0543	1.82	1.47	1.65	5.37	4.01	4.69	6.15	6.39	6.27
		NW	0.637	0.527	0.582	0.0471	0.0542	0.0507	1.80	1.49	1.65	5.89	4.27	5.08	5.92	6.29	6.11
Mean			0.538	0.429	0.484	0.0497	0.0516	0.0507	1.80	1.46	1.63	5.52	4.27	4.90	6.13	6.43	6.28
<i>M. wallichii</i>	1800 m	SW	0.456	0.385	0.421	0.0507	0.0302	0.0405	1.20	1.25	1.23	5.26	3.61	4.44	7.21	7.04	7.13
		NE	0.629	0.358	0.494	0.0606	0.0574	0.0590	1.58	1.35	1.47	6.02	5.53	5.78	6.43	6.76	6.60
		NW	0.523	0.477	0.500	0.0506	0.0421	0.0464	1.07	1.59	1.33	7.35	4.81	6.08	6.65	6.83	6.74
Mean			0.536	0.407	0.471	0.0540	0.0432	0.0486	1.28	1.40	1.34	6.21	4.65	5.43	6.76	6.88	6.82

NE- North East aspect, SW- South West aspect, NW- North West aspect

**Table 4: Main and Interaction effects of total Nitrogen (%) on *Habenaria intermedia* and *Microstylis wallichii* inhabited sites along attitude and aspect.**

**Table 4a Two factor analysis (Altitude X Depth) and (Altitude X Aspect)**

<i>Habenaria intermedia</i>								<i>Microstylis wallichii</i>							
Altitude	Depth (cm)			Aspect				Altitude	Depth (cm)			Aspect			
	0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean		0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean
2400 m	0.333	0.260	0.296	0.201	0.352	0.335	0.296	2200 m	0.473	0.324	0.398	0.380	0.412	0.403	0.398
2200 m	0.404	0.304	0.354	0.325	0.333	0.403	0.354	2000 m	0.538	0.429	0.483	0.473	0.395	0.582	0.483
2000 m	0.538	0.429	0.483	0.473	0.395	0.582	0.483	1800 m	0.536	0.407	0.471	0.421	0.493	0.500	0.471
Mean	0.425	0.331		0.333	0.360	0.440			0.515	0.387		0.425	0.433	0.495	
C.D (0.05); Altitude = 0.0243; Depth = 0.0198; Aspect & Altitude = 0.0198; Altitude X Depth = NS; Aspect X Altitude = NS								C.D (0.05); Altitude = 0.0705; Depth = 0.0576 Aspect & Altitude = 0.0576; Altitude X Depth = NS; Aspect X Altitude = NS							

**Table 4b: Two factor analysis (Aspect x Depth)**

<i>Habenaria intermedia</i>				<i>Microstylis wallichii</i>			
Aspect	Depth (cm)			Aspect	Depth (cm)		
	0-15 cm	15-30 cm	Mean		0-15 cm	15-30 cm	Mean
South west	0.372	0.295	0.333	South west	0.475	0.374	0.425
North east	0.414	0.307	0.361	North east	0.532	0.335	0.434
North West	0.488	0.392	0.440	North West	0.540	0.451	0.500
Mean	0.424	0.331		Mean	0.516	0.387	
C.D (0.05); Aspect & Depth = 0.0243; Aspect X Depth = NS				C.D (0.05); Aspect = 0.0705; Depth = 0.0576; Aspect X Depth = NS			

**Table 5: Main and Interaction effects of total phosphorus (%) on *Habenaria intermedia* and *Microstylis wallichii* inhabited sites along attitude and aspect.**

**Table 5a: Two factor analysis (Altitude X Depth) and (Altitude X Aspect)**

<i>Habenaria intermedia</i>								<i>Microstylis wallichii</i>							
Altitude	Depth (cm)			Aspect				Altitude	Depth (cm)			Aspect			
	0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean		0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean
2400 m	0.0472	0.0333	0.0403	0.0380	0.0417	0.0411	0.0403	2200 m	0.0423	0.0299	0.0316	0.0397	0.0472	0.0214	0.0361
2200 m	0.0406	0.0292	0.0349	0.0320	0.0514	0.0214	0.0349	2000 m	0.0498	0.0516	0.0507	0.0471	0.0542	0.0506	0.0506
2000 m	0.0497	0.0516	0.0507	0.0514	0.0214	0.0506	0.0411	1800 m	0.0540	0.0433	0.0486	0.0454	0.0590	0.0463	0.0502
Mean	0.0459	0.0380		0.0407	0.0382	0.0377		Mean	0.0487	0.0416		0.0441	0.0537	0.0394	
C.D (0.05); Altitude = 0.165 Depth = 0.135; Aspect & Altitude = 0.135; Altitude X Depth = NS; Aspect X Altitude = NS								C.D (0.05); Altitude = 0.0206; Depth = 0.0168; Aspect & Altitude = 0.0168; Altitude X Depth = NS; Aspect X Altitude = NS							

**Table 5b: Two factor analysis (Aspect x Depth)**

<i>Habenaria intermedia</i>				<i>Microstylis wallichii</i>			
Aspect	Depth (cm)			Aspect	Depth (cm)		
	0-15 cm	15-30 cm	Mean		0-15 cm	15-30 cm	Mean
South west	0.0453	0.0327	0.0390	South west	0.0511	0.0337	0.0424
North east	0.0507	0.0475	0.0491	North east	0.0531	0.0539	0.0535
North West	0.0415	0.0339	0.0377	North West	0.0418	0.0371	0.0368
Mean	0.0606	0.0380		Mean	0.0487	0.0416	
C.D (0.05); Aspect = 0.165; Depth = 0.135; Aspect X Depth = NS				C.D (0.05); Aspect = 0.0206; Depth = 0.0168; Aspect X Depth = NS			

**Table 6: Main and Interaction effects of total potassium (%) on *Habenaria intermedia* and *Microstylis wallichii* inhabited sites along attitude and aspect.****Table 6a Two factor analysis (Altitude X Depth) and (Altitude X Aspect)**

<i>Habenaria intermedia</i>								<i>Microstylis wallichii</i>							
Altitude	Depth (cm)			Aspect				Altitude	Depth (cm)			Aspect			
	0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean		0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean
2400 m	1.392	1.317	1.354	1.41	1.39	1.27	1.35	2200 m	1.52	1.36	1.45	1.64	1.38	1.33	1.45
2200 m	1.517	1.333	1.425	1.45	1.50	1.33	1.43	2000 m	1.81	1.46	1.64	1.61	1.65	1.65	1.64
2000 m	1.807	1.463	1.635	1.61	1.65	1.65	1.64	1800 m	1.28	1.40	1.37	1.22	1.46	1.33	1.34
Mean	1.571	1.371		1.49	1.51	1.42		Mean	1.54	1.41		1.49	1.50	1.43	
C.D (0.05); Altitude = 0.385; Depth = 0.314; Aspect & Altitude = 0.314; Altitude X Depth = NS; Aspect X Altitude = NS								C.D (0.05); Altitude = 0.460; Depth = 0.376; Aspect & Altitude = 0.376; Altitude X Depth = NS; Aspect X Altitude = NS							

**Table 6b: Two factor analysis (Aspect x Depth)**

<i>Habenaria intermedia</i>				<i>Microstylis wallichii</i>			
Aspect	Depth (cm)			Aspect	Depth (cm)		
	0-15 cm	15-30 cm	Mean		0-15 cm	15-30 cm	Mean
South west	1.583	1.393	1.488	South west	1.59	1.40	1.50
North east	1.600	1.422	1.511	North east	1.57	1.42	1.50
North West	1.532	1.298	1.415	North West	1.46	1.42	1.44
Mean	1.572	1.371		Mean	1.54	1.41	
C.D (0.05); Aspect = 0.385; Depth = 0.314; Aspect X Depth = NS				C.D (0.05); Aspect = 0.460; Depth = 0.376; Aspect X Depth = NS			

**Table 7: Main and Interaction effects of total Organic carbon (%) on *Habenaria intermedia* and *Microstylis wallichii* inhabited sites along attitude and aspect.****Table 7a Two factor analysis (Altitude X Depth) and (Altitude X Aspect)**

<i>Habenaria intermedia</i>								<i>Microstylis wallichii</i>							
Altitude	Depth (cm)			Aspect				Altitude	Depth (cm)			Aspect			
	0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean		0-15 cm	15-30 cm	Mean	South West	North East	North West	Mean
2400 m	4.409	3.717	4.063	3.470	3.180	5.539	4.063	2200 m	4.92	3.85	4.38	3.87	5.19	4.09	4.38
2200 m	4.070	3.628	3.849	3.090	4.365	4.093	3.849	2000 m	5.52	4.27	4.90	4.92	4.69	5.08	4.90
2000 m	5.523	4.263	4.895	4.915	4.692	5.079	4.895	1800 m	6.21	4.65	5.43	4.44	5.78	6.08	5.43
Mean	4.670	3.870		3.830	4.080	3.057		Mean	5.55	4.25		4.41	5.22	5.08	
C.D (0.05); Altitude = 0.703; Depth = 0.574; Aspect & Altitude = 0.574; Altitude X Depth = NS; Aspect X Altitude = NS								C.D (0.05); Altitude = 1.18; Depth = 0.97; Aspect & Altitude = 0.97; Altitude X Depth = NS; Aspect X Altitude = NS							

**Table 7b: Two factor analysis (Aspect x Depth)**

<i>Habenaria intermedia</i>				<i>Microstylis wallichii</i>			
Aspect	Depth (cm)			Aspect	Depth (cm)		
	0-15 cm	15-30 cm	Mean		0-15 cm	15-30 cm	Mean
South west	4.160	3.490	3.852	South west	4.99	3.83	4.41
North east	4.420	3.738	4.079	North east	5.78	4.66	5.22
North West	5.422	4.385	4.904	North West	5.89	4.28	5.09
Mean	4.667	3.871		Mean	5.55	4.27	
C.D (0.05); Aspect = 0.703; Depth = 0.574; Aspect X Depth = NS				C.D (0.05); Aspect = 1.18; Depth = 0.97; Aspect X Depth = NS			



**Table 8: Ecological variables in relation with *Habenaria intermedia* and *Microstylis wallichii* at different altitude and aspects with relation to associates and soil physico- chemical properties**

<b><i>Habenaria intermedia</i></b>									
<b>Altitude</b>	<b>2000 m</b>			<b>2200 m</b>			<b>2400 m</b>		
<b>Aspect</b>	<b>SW</b>	<b>NE</b>	<b>NW</b>	<b>SW</b>	<b>NE</b>	<b>NW</b>	<b>SW</b>	<b>NE</b>	<b>NW</b>
<b>Herbs, grasses &amp; seedlings</b>									
<b>Total species</b>	53	35	43	36	46	54	26	40	38
<b>Total density (plants/m<sup>2</sup>)</b>	131.17	105.10	151.40	171.00	176.60	208.60	128.70	144.50	154.10
<b>Shrub, climbers &amp; saplings</b>									
<b>Total species</b>	25	18	16	8	8	10	10	9	8
<b>Total density (plants /25m<sup>2</sup>)</b>	36.30	28.00	18.9	16.70	22.30	43.60	41.40	32.00	15.60
<b>Trees</b>									
<b>Total species</b>	8	8	7	0	0	6	0	4	4
<b>Total density (plants /100m<sup>2</sup>)</b>	23.70	13.3	14.40			12.60		3.6	2.20
<b><i>Habenaria intermedia</i></b>									
<b>Total density (plants /m<sup>2</sup>)</b>	4.90	3.40	2.1	7.30	4.30	3.70	5.2	4.60	6.40
<b>Total basal cover (cm<sup>2</sup>/m<sup>2</sup>)</b>	3.10	0.82	0.55	1.69	1.94	1.04	0.66	1.16	1.21
<b>IVI</b>	9.80	10.84	5.29	12.70	12.26	8.82	10.83	17.83	17.52
<b><i>Microstylis wallichii</i></b>									
<b>Altitude</b>	<b>1800 m</b>			<b>2000 m</b>			<b>2200 m</b>		
<b>Aspect</b>	<b>SW</b>	<b>NE</b>	<b>NW</b>	<b>SW</b>	<b>NE</b>	<b>NW</b>	<b>SW</b>	<b>NE</b>	<b>NW</b>
<b>Herbs, grasses &amp; seedlings</b>									
<b>Total species</b>	49	52	42	53	35	43	46	50	54
<b>Total density (plants/m<sup>2</sup>)</b>	187.40	169.90	15.20	131.17	105.10	151.40	173.70	157.30	208.60
<b>Shrub, climbers &amp; saplings</b>									
<b>Total species</b>	13	17	13	25	18	16	18	21	10
<b>Total density (plants/25m<sup>2</sup>)</b>	24.80	21.60	17.50	36.30	28.00	18.9	55.80	30.90	43.60
<b>Trees</b>									
<b>Total species</b>	4	8	4	8	8	7	4	7	6
<b>Total density (plants/100m<sup>2</sup>)</b>	9.8	9.8	9	23.70	13.3	14.40	11.20	12.60	12.60
<b><i>Microstylis wallichii</i></b>									
<b>Total density (plants/m<sup>2</sup>)</b>	27.10	11.70	33.50	9.00	9.40	16.30	21.00	12.50	9.70
<b>Total basal cover (cm<sup>2</sup>/m<sup>2</sup>)</b>	13.77	36.99	65.74	23.40	7.41	31.87	15.80	15.65	6.83
<b>IVI</b>	38.12	65.50	94.34	29.85	40.50	64.92	40.99	27.62	34.93

NE- North East aspect, SW- South West aspect, NW- North West aspect

#### 4. Conclusion

The physico-chemical soil attributes associated with *H. intermedia* and *M. wallichii* in different altitude and aspect will provide valuable information for *in situ* and *ex situ* conservation of orchid species as they reflect the suitability of site according to density of the species. *H. intermedia* require proper aeration and less moisture around their roots. *M. wallichii* prefer damp cool habitat with high moisture content but require good aeration for superficial root system spread on litter layer. Increase in nitrogen, potassium and organic carbon content with decrease in altitude also limits the density of *H. intermedia* while phosphorus with increase in altitude increases the density. Nitrogen and organic carbon was low in south

west aspect compared to other aspect and also support the relatively high density of *H. intermedia*. In case of *Microstylis wallichii* the high density of species was observed with respect to high nitrogen and organic carbon. Further, general observation suggest that *H. intermedia* prefer the open grasslands and soil exposed slopes while *M. wallichii* prefers the shady places under Oak and Oak mixed forests. In cultivation promotional programme these information may be used in manipulation of nutrients requirement and other soil characteristics for ensuring the success of cultivation.

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