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Soil Treatments for Improving Seed Germination of Rare and Endangered Sikkim Himalayan Rhododendrons

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Abstract: A field survey was conducted to find out the status of the availability of rhododendrons of Sikkim Himalaya. Rhododendrons, denizens of high altitude, have a characteristic slow growth rate. Seeds of rhododendron are clearly distinguished from their outer micro-and macro-morphological variability from species to species. Seed germination of 10 elite species of rhododendrons were investigated in various treatments using rhododendron rhizosphere soil (Ratey Chuu, East Sikkim,), non-forest soil and sterilized soil in various combinations. Results obtained from various soil treatments indicated the presence of certain factors probably of microbial origin influencing the seed germination. A total of seven soil treatments taken under consideration were: (T1) sterilized soil, (T2) forest soil, (T3) degraded soil, (T4) rhizosphere natural soil and (T5, T6, T7) rhizosphere natural soil: degraded soil in three ratios (1:3, 1:1, 3:1), respectively. Best results on per cent seed germination were recorded in the treatment where rhododendron rhizosphere soil was mixed with degraded soil in 1:1 ratio followed by rhododendron rhizosphere soil mixed with degraded soil in 3:1 ratio. Soil respiration, total organic carbon, phosphorus and nitrogen varied significantly between soil types. Chlorophyll a and total chlorophyll content of rhododendron species were always higher in rhododendron rhizosphere soil based treatments compared to other soil treatments. Mean net photosynthetic rate of rhododendron seedlings was greater in rhododendron rhizosphere soil based treatments. Photosynthesis varied significantly between species grown in different soil types.

Key words: Rhododendrons · Chlorophyll · Photosynthesis · Rhizosphere soil · Seed germination

INTRODUCTION

Rhododendrons (Family-Ericaceae) are extremely hardy evergreen flowering shrubs and trees of subtropical and temperate origin. Rhododendrons are the denizens of high altitude, comprising about 1000 species mainly inhabiting a vast section of South-Eastern Asia stretching from the North-Western Himalaya through Nepal, Sikkim, Eastern Tibet, Bhutan, Arunanchal Pradesh, Northern Myanmar and Western and Central China; more than 90% of the worlds population of rhododendrons belong to this region [1].

The genus *Rhododendron*, having about 50 species in India [2-4], is mainly distributed in the Himalayan region (one species *R. nilagiricum* Zenk. grows in South India). Out of these, about 36 species with 45 different forms including subspecies and varieties occur in Sikkim alone. The genus forms a very important dominant combination of forest types in the cool temperate and subalpine region and also on the alpine meadows of the Sikkim Himalaya, as about 72% of the Indian species are found in Sikkim. Therefore, it is considered as one of the most appropriate locations to undertake conservation and propagation studies of rhododendrons in India [5-7]. The major threats for survival of this genus are deforestation, unsustainable extraction for firewood and incense by the locals. The rise in human population with demand on land for farming, increased number of livestock, construction of roadways, hydel power stations and allied works, army personals garrisoned at alpine locations and lately the tourist influx over the nature trails have collectively resulted in the building up of considerable pressure on the availability of the rhododendron species.

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The microbial biomass constitutes a very important component of soil organic matter since it can sensitize to any change in organic inputs [8]. Interactions between plants and soil microorganisms have long been recognized for their importance in plant mineral nutrition and nutrient cycling. Mycorrhizae are beneficial for the growth because of the enhanced nutrient uptake. Soil biomass, a labile fraction of soil organic matter [9]. is a major nutrient sink during immobilization and a source during mineralization. Nutrient level in microbial biomass is much influenced by soil physicochemical properties. Soils with a relatively high organic matter input usually develop a larger microbial biomass. Arbuscular mycorrhizal fungi found in a range of host plants may play an essential ecological role in highly stressed environments, such as arctic and alpine habitats, where plant communities experience short growing period, low temperature, low nutrient status and low decomposition rates. Physical parameters, such as light and temperature, also determine the success or failure of a species on a particular locality, which in turn depends mostly on the germinability of the seeds of a particular species. Rhododendrons in Sikkim usually grow under a moist environment with plenty of soil moisture, humidity and at the locations above 2800 m. The winter season with severe drop in temperature at localities above 2800 m produces permafrost type situation in the soil. A physiological drought condition is thus always experienced by these plants at such locations resulting in thwarted growth.

Very little information is apparently available on the germination of rhododendrons. So far, no long term storage methods have been suggested for the seed of rhododendrons, therefore it is important to examine their desiccation response in order to obtain preliminary information for their seed germination and its conservation. It is now widely accepted that the physiological quality of seed, defined in terms of percentage, rate and uniformity of germination, has a major impact on the efficiency and production. Physical parameters such as light and temperature also determine the success or failure of a species on a particular locality, which in turn depends mostly on the germinability of the seeds of a particular species.

The regeneration of rhododendrons in nature is through seed only which is reportedly very low. The role of rhizoflora, positive or negative on seed germination and subsequent plant growth of some important tree species of Indian Himalayan region has been reported recently [10, 11]. In the present study the role of rhizoflora (in various soil combinations) on seed germination of 10 species of rhododendrons of Sikkim Himalaya has been investigated. In addition, the influence of these soil combinations on various physiological growth parameters has also been studied.

MATERIALS AND METHODS

Field Survey: At the outset of the study, a field survey of North (Singba, Yumthang, Lachung, Lachen), East (Tsangu and Kukup) and West Sikkim (Dzongri) was conducted to find out the status of the rarity of rhododendrons in Sikkim.

Study Site: Experiments on seed germination of rhododendrons were conducted under polyhouse conditions in the Institute's nursery (Longitude 27°4'46" to 28°7'48" North and Latitude 88°55' to 88°55'25" East with an elevation 2087 m amsl at Pangthang. The climate of the Pangthang area divisible into three seasons is: winter (November-February), spring (March-May) and rainy (June to September). The annual rainfall varies between 3000-4000 mm, of which more than 80% occurs in the rainy season. The average maximum and minimum temperature during year varies from 19-27°C and 4-20°C, respectively. Relative humidity varied between 80 to 95% during the rainy season and decreased to about 45% in spring.

Seed and Soil Collection: Seed of 10 available species of rhododendrons were collected from different localities situated between an altitude 2500 to 4500 m amsl in the Sikkim Himalaya. As the period of seed maturity varied with species, the seeds were collected between September and December. These were then dried in the open air for one week and then stored in small bottles/polythene bags in the laboratory under natural light and temperature conditions. Observations on the morphological features, such as seed length and seed width, were recorded prior to sowing. For making different treatments, soil was collected from different locations. The forest soil was collected from Pangthang arboretum, rhizosphere soil from Ratey Chuu (East Sikkim) and degraded soil from new building site of the Institute. For sterilized soil treatment, the rhododendron rhizosphere soil autoclaved thrice for ½ h at three consecutive days was used.

Experimental Set Up: Seeds of 10 species of rhododendrons were sown in 7 types of soil combinations. One hundred (approximate) air-dried seeds (in triplicate) for each species were used. The treatments under consideration were: (T1) sterilized soil, (T2) forest soil, (T3) degraded soil, (T4) rhododendron rhizosphere soil, (T5) rhododendron rhizosphere soil mixed with degraded soil in 1:3 ratio, (T6) rhizosphere soil of rhododendron mixed with degraded soil in 1:1 and (T7) rhododendron rhizosphere soil mixed with degraded soil in 3:1. The seeds were sown in thermacol cups and placed in a polyhouse at Panthang. The cups were placed inside a greenhouse at Pangthang at the altitude 2087 m amsl. Seedlings were raised under 30-70 µmol m^{-2} s^{-1} light intensity with temperature ranging from 24°C to 12°C and mean relative humidity 85% during the study period. The cups were watered and weeded regularly. Germination was recorded after one year.

Soil Analyses: Soil pH for each treatment was measured with a glass electrode pH meter in a soil and water mixture in 1:2 ratio. Soil samples were dried, ground to pass through a 2 mm sieve and used for nutrient analysis. Total nitrogen was estimated by a modified Kjeldahl method [12] and total phosphorus by using hydrogen peroxide oxidized acidified ammonium fluoride extract by chlorostannous reduced molybdophosphoric blue colour method [13].

Soil Respiration: Soil respiration was measured with a CO_2 Infrared Gas Analyzer (CI-301 PS Model, USA) equipped with a soil respiration chamber that fits on top of the soil and were generally conducted in the morning between 0800 and 1200 hrs. Chamber consisted of 0.65 liter as the chamber volume and 74.5 cm^2 as the leaf area.

Net Photosynthetic Rate and Photosynthetic Active Radiation (PAR): Net photosynthetic rate and incident PAR (wave band 400-700) of rhododendron seedlings grown in different soil combinations were measured during the growing season. All measurements were conducted under sunny skies between 1100 and 1400 hours. Leaf photosynthetic rate and PAR were measured using a portable CO_2 gas analyzer (LCD-Spectra, USA).

RESULTS AND DISCUSSION

Field Survey and Seed Morphology: Based on the field survey, out of 36 species of Sikkim Himalayan rhododendrons, 10 were selected (with 45 different forms including sub-species and varieties) for the present study. The results of the field survey showing the rarity of these species are presented in Table 1. Seeds of all the investigated species of rhododendrons were small and consisted of an oval to oblong endosperm enclosed within a transparent fusiform to oblong thin testa. The seeds ranged from 0.67 to 3.12 mm in length and from 0.29 to 0.93 mm in width. The endosperm length ranged from 0.53 to 1.47 mm. Some of the observations on seed morphology have been published earlier [14]. The anticlinal walls were thicker than the outer periclinal walls of the testa cells, giving a characteristic mask-like appearance. Seed dimensions among the observed samples of each species varied (Table 2). The surface of the cells of the testa (the outer periclinal walls) was rough and variously pitted. SE micrographs clearly showed the rough and pitted patterns located on the outer periclinal walls. Variability in the pitting patterns within each sample was recorded. However, pitting patterns showed systematic value. Wide variation in the seed dimensions for both macro and micro-morphological features were observed. The capsules of *R. dalhousiae*, also associated with the large seed size, were larger in diameter than those of other species.

Seed Germination Between Soil Treatments: Seed germination began in the middle of June in all the species of rhododendrons in all the soil treatments. Data recorded for 10 species of rhododendrons after 12 months of sowing exhibited wide range in per cent seed germination. Minimum seed germination (0.67%) was recorded in *R. setosum* in rhododendron rhizosphere sterilized soil and forest soil treatments, which reached upto 3.33% in rhododendron rhizosphere natural soil combined with

Table 1: Rarity of species in the Sikkim Himalaya

	Availability			Space			
Species	Few	Extremely few	Large	Ubiquitous	Localized	Extremely localized	Status
R. grande				+			Threatened
R. setosum			+		+		Threatened
R. dalbousiae	+			+			
R. ciliatum	+					+	
R. pendulum		+			+		Rare
R. maddeni		+			+		Rare
R. thomsonii			+		+		Vulnerable
R. lepidotum	+				+		
R. niveum		+				+	Very rare
R. baileyi	+					+	Threatened

Table 2: Morphological features of the seeds of rhododendrons

Species	Habitat and distribution	Flowering time	Fruiting time	Mean seed length (l) (mm)	Mean seed width (w) (mm)	Ratio (l/w)
R. grande Wt. Large silvery Rhododendron N-Patle Korlinga	Common in Sikkim Himalayas, tree, distributed from E. Nepal, Sikkim, Darjeeling, Bhutan to S.E. Tibet at 2000-3000	Mar-Apr	Nov	8.81	4.75	1.85
R. setosum D. DonBristly RhododendronN-Tsallu Guras	Shrub, Highly aromatic, open rocky situations gregariously and growing in association with other high altitude species like R. nivale, R. lepidotum, etc., distributed from Eastern Nepal, Sikkim, Bhutan and S.E. Tibet at 3000-5500	Jun-Jul	Oct-Nov	3.32	1.59	2.08
R. dalhousiae Hook.Lady Dalhousie's Rhododendron N - Lahare Chimal	Parasitical on the trunks of large trees of Michelia and Quercus; Nepal, Sikkim, Darjeeling, Bhutan and Arunachal Pradesh at 1500-2500m	Apr-May	Nov	3.12	0.74	4.21
R. ciliatum Hook.Ciliated RhododendronN-Junge Chimal	Marshy situations, well exposed to sunlight; Lachung and Lachen in Northeast Sikkim, Eastern Nepal through Sikkim, Bhutan and S.E. Tibet at 3000-3800m	Apr-May	Oct	1.74	0.45	3.90
R. pendulum Hook. f. Pendulous Rhododendron N.-Jhundinae Chimal	Abies forest epiphytic shrub and pendulous from trees of Abies spectabilis and A. brunonianae, distributed in Lachung and Yumthang in North Sikkim, E. Nepal and S.E. Bhutan at 3300-4000m	Apr-May	Oct	3.89	0.88	4.42
R. maddeni Hook.Major Madden's Rhododendron N-Major Madden ko Chimal	Rocky valleys and ridges growing; Yakchey in North Sikkim; endemic to Bhutan and Sikkim	Jun-Aug	Nov	2.54	0.49	5.18
R. thomsoni Hook. Dr. Thomson's Rhododendron N-Dr. Thomson's ko Gurans	Open situations or forms mixed shrubberies with other Rhododendron; Nepal, Sikkim, Bhutan, Darjeeling and S.E. Tibet at 3300-4500m	May-Jun	Nov	1.71	0.52	3.90
R. lepidotum Wall.ex G. DonScaly Rhododendron N-Bhale Sunpate	Open rocky situations; Western and Central Himalaya to S.W. China at elevations of 2500-4500m and in Sikkim at 2500-5000m	Jun-Jul (to Oct)	Nov-Dec	0.67	0.29	2.31
R. niveum Hook.Snow-leaved Rhododendron N-Hiun-pate Gurans	Rocky valleys and ridges growing; Yakchey in North Sikkim; endemic to Bhutan and Sikkim at 3500-4500m	Apr	Oct	1.42	0.60	2.36
R. baileyi Balf.f. Bailey's Rhododendron N-Bailey Ko Chimal	Terrestrial evergreenShrub, distributed in Tibet, Bhutan and Sikkim at 3000-4800	May-Jun	Oct	1.46	0.71	2.05

Table 3: Percent seed germination of rhododendrons using various soil treatments

	Seed germination (%)						
Name of species	T 1	T 2	T 3	T 4	T 5	T 6	T 7
R. grande	6.00±1.63	4.33±0.72	3.00±0.47	3.67±0.98	2.33±0.27	4.67±0.54	4.33±0.72
R. setosum	0.67±0.54	0.67±0.54	1.33±0.54	2.00±0.94	2.67±1.19	3.33±1.09	2.33±0.98
R. dalhousiae	29.67±1.66	27.33±1.78	27.00±1.25	36.33±1.44	28.00±0.94	36.33±0.72	31.00±2.16
R. ciliatum	27.67±0.98	20.00±0.94	42.67±3.03	40.33±0.72	57.67±1.19	66.00±1.70	62.33±1.19
R. pendulum	21.33±1.09	21.00±0.47	22.00±1.89	26.67±1.96	27.33±3.03	44.60±0.72	40.00±1.20
R. maddeni	22.67±1.19	16.00±2.50	16.67±1.96	26.00±0.47	33.00±0.94	32..67±1.44	30.67±0.54
R. thomsoni	5.00±0.47	6.67±1.44	2.00±0.82	4.00±1.25	6.33±0.72	11.33±0.72	11.00±0.47
R. lepidotum	22.67±1.18	26.00±2.86	26.33±2.16	29.00±0.47	31.00±1.70	29.33±1.44	30.33±1.19
R. niveum	20.67±1.44	23.33±1.96	22.67±3.03	26.67±1.44	32.00±1.20	28.33±1.52	27.33±2.60
R. baileyi	12.00±0.94	10.67±0.54	16.00±0.47	16.00±2.50	16.33±1.36	18.00±0.94	19.33±1.78

T1: Rhododendron rhizosphere sterilized soil, T2: Forest soil, T3: Degraded soil, T4: Rhododendron rhizosphere natural soil, T5: Rhododendron rhizophere natural soil: degraded soil (1:3), T6: Rhododendron rhizosphere natural soil: degraded soil (1:1) and T7: Rhododendron rhizosphere natural soil: degraded soil (3:1)

degraded soil in 1:1 ratio treatment. The maximum seed germination (66.00%) was recorded in *R. ciliatum* in rhododendron rhizosphere natural soil combined with degraded soil in 1:1 ratio. In general, in almost all the rhododendron species, the three treatments where the rhododendron rhizosphere natural soil was mixed with degraded soil in various ratios, increased the per cent seed germination positively. Out of the three ratios, highest seed germination was recorded in rhizosphere natural soil mixed with degraded soil treatment in 1:1 ratio (Table 3). Mixing of degraded soil in rhododendron rhizosphere soil affected the seed germination positively

Table 4: Chemical and Physical properties and soil respiration in various soil type

Soil type	Soil texture	Bulk density (g/cm^3)	Total organic Carbon C ($mg\ g^{-1}$)	N ($mg\ g^{-1}$)	P($mg\ g^{-1}$)	pH	WHC	Soil respiration ($\mu molm^{-2}\ s^{-1}$)
Rhododendron rhizosphere sterilized soil (T1)	Sandy loam	0.96±0.062	23.3±0.72	0.371±0.034	0.214±0.014	5.10±0.054	69.88±0.108	-
Forest soil (T2)	Sandy loam	1.33±0.012	7.33±0.98	0.430±0.015	0.279±0.025	5.55±0.022	74.67±0.073	7.31±0.056
Degraded soil (T3)	Silty loam	1.30±0.305	20.00±0.94	0.390±0.167	0.388±0.022	5.70±0.047	70.65±0.184	6.61±0.138
Rhododendron rhizosphere natural soil (T4)	Sandy loam	0.90±0.047	23.66±0.72	0.381±0.035	0.298±0.028	5.00±0.043	69.33±0.467	7.53±0.118

in most of the species. Forest or degraded soil alone resulted in poor seed germination in comparison to mixed soil treatments. Sterilization of rhododendron rhizosphere soil resulted in decreasing the per cent seed germination in case of 8 species of rhododendrons. Analysis of variance for seed germination showed significant variation between soil type ($F_{5,12}$=25, P<0.0001). Tukey's pairwise comparison probabilities showed significant variation between: T5 with T6 (P<0.02); T6 with T7 (P<0.06).

The rhizosphere which is often characterized with stimulated microbial activity provides a natural consortium of microorganisms and may be used as a source of inoculum for raising healthy seedlings of forest species. Besides edaphic and climatic factors, the individual rhizosphere is affected by several other factors like the quantity and quality of root exudates secreted by a particular plant species. The effect of these biotic as well as abiotic factors is likely to be more intense, stimulatory or inhibitory, particularly in case of perennial trees. A tree rhizosphere is likely to develop a microenvironment continuously under the effect of the root exudates, soil characteristics and climatic factors, giving an opportunity for development of a specialized rhizoflora. The rhizosphere of *Rhododendron campanulatum*, a species of sub alpine Himalaya of Kumaun (Uttarakhand) region was found to exert a suppressive effect on soil microflora and causing acidity [15]. Such rhizosphere (e.g., tea) has been reported to be colonized by greater antagonistic populations providing a natural site for isolation and selection of promising microbial inoculants [16]. Diversity and colonization of arbuscular mycorrhizal fungi associated with all the five species of rhododendrons found on an altitudinal range from 1500 to 4500 m amsl in Kumaun region of Indian Central Himalaya have recently been studied [17]. In the cited study genus *Glomus* is reported to dominate the rhizosphere of all the five rhododendron species. Mycorrhizae are essential for the survival and growth of rhododendron roots as they enhance mineral and water acquisition significantly [18].

The effect of the rhizosphere soil associated with a particular species on seed germination could be influenced by mixing the soil with non forest soil in an appropriate ratio. In recent studies, combinations of different types of soil, such as forest/ non-forest soil in different ratios have been evaluated for influencing the seed germination and subsequent growth of important forest species. The seed germination in *Taxus baccata* which is reported to be very low (around 8%) increased upto 70% in a non forest soil treatment under a polyhouse [11]. Rhizosphere mixed with non-forest soil in 1:9 ratio improved the seed germination and subsequent growth of *Cedrus deodara* and *Pinus wallichiana* under nursery conditions [10]. Seed germination refers to the resumption of metabolic activities upon imbibitions of seeds followed by radical emergence and subsequent seedling growth. Small seeds, like rhododendrons require suitable medium supplemented with nutrient and carbohydrates source for vigorous *in vitro* seed germination and growth [19]. The use of plant growth substances and chemical to break dormancy and to synchronize seed germination is well known [20, 21].

The pH values in soil ranged from 5.0 and 5.7. In most cases the pH of rhizosphere soil was lower than that of non rhizosphere soil (Table 4). The pH in acidic range has been reported to support the colonization of arbuscular mycorrhizal fungi in rhododendrons [17]. The arbuscular mycorrhizal fungi are widely distributed common soil fungi being abundant in phosphorus and other mineral deficient soils and also obligate symbionts which affect the host plant positively. Moisture content was highest in forest soil followed by degraded soil and the lowest value was found in rhizosphere soil probably due to very poor water holding capacity. Soil organic carbon in the

Table 5: Effect of soil treatment on moisture, chlorophyll contents and photosynthesis of rhododendron leaves grown in different combination of soil

Species	Moisture content		Chlorophyll Content(Mg Chl-1 g-1 fresh weight) T1			T6			Photosynthesis (μmol m^{-2} s^{-1})	
	T1	T6	Chl a	Chl b	Total Chlorophyll	Chl a	Chl b	Total Chlorophyll	T1	T6
R. grande	78.42±1.18	64.22±1.66	1.37±0.049	0.53±0.036	1.90±0.023	1.41±0.024	0.78±0.001	2.19±0.028	5.35±0.271	6.83±0.072
R. setosum	73.67±2.42	71.67±1.60	1.15±0.033	0.50±0.047	1.65±0.070	1.16±0.019	0.60±0.024	1.76±0.037	4.80±0.187	5.60±0.071
R. dalbousiae	72.00±1.41	69.98±1.23	1.14±0.034	0.74±0.025	1.88±0.030	1.49±0.045	0.82±0.002	2.31±0.045	5.58±0.224	6.95±0.078
R. ciliatum	73.43±1.95	70.64±1.42	1.49±0.023	0.79±0.060	2.28±0.280	1.68±0.011	0.98±0.004	2.66±0.012	6.10±0.169	7.15±0.014
R. pendulum	74.37±2.20	69.13±1.78	0.95±0.029	0.443±0.028	1.39±0.011	1.08±0.018	0.52±0.016	1.60±0.031	4.80±0.324	5.75±0.235
R. maddeni	71.66±1.33	67.29±1.73	1.13±0.012	0.72±0.041	1.85±0.053	1.24±0.015	0.71±0.012	1.95±0.026	5.10±0.052	6.80±0.076
R. thomsonii	75.41±2.38	70.96±1.59	1.12±0.012	0.81±0.033	1.93±0.027	1.55±0.021	0.85±0.012	2.40±0.024	5.25±0.053	6.80±0.108
R. lepidotum	77.37±1.07	70.00±0.89	1.09±0.053	0.67±0.012	1.76±0.049	1.24±0.034	0.66±0.026	1.90±0.054	5.80±0.024	6.72±0.098
R. niveum	70.00±1.25	60.76±1.73	1.23±0.031	0.87±0.028	2.10±0.040	1.48±0.042	0.83±0.033	2.31±0.038	5.43±0.093	6.78±0.229
R. baileyi	68.17±1.39	67.59±0.98	0.92±0.015	0.54±0.040	1.46±0.034	1.16±0.031	0.54±0.310	1.70±0.062	4.65±0.317	5.62±0.181

T1. Rhododendron rhizosphere sterilized soil, T6. Rhododendron rhizosphere natural soil: garden soil (1:1)

different combination of soil varied significantly. The highest value was recorded in the forest and the lowest in the degraded soil (Table 4). Total nitrogen was higher in the forest soil followed by degraded soil and the lowest in rhizosphere soil. Total phosphorus in different soil combination ranged from 0.388 mg g^{-1} soil in degraded soil to 0.279 mg g^{-1} soil in forest soil. Organic phosphorus was recorded highest in forest soil and lowest in degraded soil. It has now been established that phosphorus is extremely essential for the proper growth of the seedlings of rhododendrons [22].

Soil Respiration: Mean rates of soil respiration varied widely within and among soil types. The highest rate of soil respiration 7.53 μmol m^{-2} s^{-1} occurred in the rhizosphere soil, where as soil respiration in degraded soil and forest soil ranged from 6.61 to 7.31 μmol m^{-2} s^{-1} (Table 4). Soil respiration rates vary significantly among major plant biomes, suggesting that vegetation type influences the rate of soil respiration [23]. Soil potentially influencing rates of soil respiration *in situ* include the availability of C substrates for microorganisms [24], plant root densities and activities [25] and soil organism population levels [26]. Plants produce the organic matter that feed soil organisms and soil biota transform organically bound nutrients into forms that can be utilized by plants [23]. Analysis of variance for soil respiration showed significant variation between soil type ($F_{5,12}$=25, P<0.0001). Significant correlation of Total organic carbon were found with Phosphorous [Total Organic carbon = 0.322 + 0.073 Phosphorous; R=0.584, $F_{1,21}$=10.8, P<0.005]. Nitrogen did not show any significant correlation with soil respiration values.

Chlorophyll Content and Net Photosynthesis: The results showed that during the growing season photosynthetic active radiation (PAR) was commonly below 70 μmol m^{-2} s^{-1} (0.25% of full sunlight) during day light under the polyhouse. Other aboveground factors (temperature and humidity) were not likely to be important because they did not differ significantly between forest types. The results showed that light was an important resource for tree seedlings growing in forest harboring rhododendrons, but other resources such as soil water content may be equally or more important than light (data not shown).

Total chlorophyll contents were relatively higher in leaves of rhododendron grown in rhizosphere soil mixed with degraded soil in 1:1 ratio than in the sterilize soil (Table 5). Total chlorophyll of rhododendron ranged from 1.60-2.66 mg g^{-1} fresh weight in rhizosphere soil mixed with degraded soil in 1:1 ratio to 1.39-2.28 mg g^{-1} fresh weight in sterilized soil. Variation between soil types was statistically significant. Chlorophyll a in rhododendron leaves was always higher than chlorophyll b. Chlorophyll a varied significantly between species and soil type, while chlorophyll b content was significant only between species. The results showed that light was an important resource for tree seedlings growing in forest harboring rhododendrons, but other resources such as soil water content may be equally or more important than light.

Net photosynthetic rate was on average almost twice as great when rhododendron grown in the rhizosphere soil mixed with degraded soil based treatments as compare in the sterilized soil. Rhododendron species grown in rhizosphere soil mixed with degraded soil showed significant increase in photosynthetic rate while same rhododendron species when grown in sterilized soil, net photosynthesis were observed in lower rate. Mechanisms that might be responsible by rhizosphere soil, enhanced soil nutrient availability, or enhanced soil water availability among seedlings. Most commonly the relationship between nitrogen status and photosynthesis has been investigated by manipulating the nitrogen availability. Photosynthesis-nitrogen relationships are intrinsically complex, because photosynthesis represents the integrated operation of a series of processes sensitive to environmental factors as well as leaf physiology and structure [27]. Nitrogen is commonly stored by plants in the form of RuBP carboxylase [28], thus the proportion of leaf nitrogen in RuBP carboxylase commonly increases with increasing leaf nitrogen concentration [29]. As leaf nitrogen concentration increases the photosynthetic rate also increases for which the photon flux densities are required to make efficient use of high leaf nitrogen concentration [30-32]. In the present study, total chlorophyll and chlorophyll a showed positive correlation with nitrogen content of the leaf consistent with the above report as chlorophyll is the site of photosynthesis. Rhododendron grows in a diversity of moist habitats in which photosynthetic photon flux density and temperature are the most important environmental factors. Many plants exude or deposit a substantial portion of their photosynthate produced in the canopy into the rhizosphere. Photosynthesis of rhododendrons varied significantly between soil type ($F_{5,12}$=456, P<0.0001). Tukey's pairwise comparison probabilities were significant between rhizosphere soil and rhododendron rhizosphere natural soil: garden soil (P<0.0001). Moisture content did not show any significant correlation with soil type.

On the basis of above observations, it may be concluded that the germination percentage of most of the species of rhododendrons can be influenced by using various soil combinations. The mixing of rhododendrons rhizosphere soil with degraded soil in an appropriate ratio (1:1 or 1:3) can be recommended for improving the seed germination and subsequent seedling growth. The study has implications for afforestation (as well as reforestation) programmes where the availability of a large number of healthy seedlings at nursery level is a prerequisite. An appropriate amount of rhizosphere soil preferably from the corresponding taxa, may carry all the microorganisms including mycorrhizae and prove to be an excellent source of "inoculum" to colonize nutritionally poor soils at degraded sites.

Research initiatives on regeneration of rhododendrons: Our Institute (GBPIHED) has been evaluating the status of the rhododendrons in nature for the past 10 years and *ex situ* regeneration work is in progress. Biotechnological research on *R. maddeni* has been supported by central funding agencies since 1999 but still more work is required to reach the next level of *in vitro* programme, particularly all endangered and threatened Sikkim Himalayan rhododendron, i.e., complete establishment of proliferation in culture. In nature, the plant is a very slow growing woody shrub. Higher polyphenols and flavonoids exudation from the explants makes the sterilization and proper establishment of explants difficult. An *ex situ* conservation initiative of the Institute has been establishment of a Rhododendron Arboretum at Pangthang near Gangtok in Sikkim. Already twenty-four species of rhododendrons are housed here and some of them have started flowering. This is the only *ex situ* conservation initiative on rhododendrons in India. The rare and endangered species mentioned in Table 1 are in process of mass propagation by the Institute for their restoration in nature in near future. Some of the rare and endangered species are now under *in vitro* research procedures.

Under a collaborative programme between G.B. Pant Institute of Himalayan Environment and Development, Sikkim unit (Pangthang-Gangtok) and Forest, Environment, & Wildlife Management Department, Government of Sikkim' (approved proposal from PCCF), a unique kind of rare and threatened plant Conservation Park, covering 2 ha area was established in the Himalayan Zoological Park, Bulbulay-Gangtok. This has targeted the plantation of over a large number of high quality, tissue culture and nursery raised rare and threatened rhododendron plants, developed and contributed by the G.B. Pant Institute. One hundred tissue cultured raised *R. maddeni* (three years old) and one hundred fifty conventional plants of rhododendron species; *R. griffithianum, R. baileyii, R. grande, R. dalhousiae* and *R. cilatum* were transplanted at field sites where they are growing well. This is a joint venture of above two departments, in which the plant species including plantation of target species will be monitored by the GBPIHED, Sikkim unit on regular basis while the management aspect will be looked after by the Department (Zoo Authorities) of Forest, Government of Sikkim. The goal of the above work is to find out means of conservation through *in vitro* and *ex situ* mass propagation and restoration of rhododendron population in the wild.

In addition, initiatives on "rhizosphere microbiology" related aspects of rhododendrons of Uttarakhand have also been taken up at GBPIHED, Almora. Microbial inoculants have been developed and the microbiological interventions will be included in propagation packages being developed for conservation of rhododendrons.

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