STUDY OF LEAF FUNCTIONAL TRAITS IN *QUERCUS SEMICARPIFOLIA* AND *QUERCUS LANATA* AT ALTITUDINAL GRADIENT IN SHIVAPURI NAGARJUNA NATIONAL PARK, KATHMANDU, NEPAL

A THESIS

SUBMITTED FOR THE
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE MASTER'S DEGREE IN (BOTANY)

BY

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DEDICATION

This study is dedicated to my parents, people and all the ecologist who have supported me and provide guidance to complete my education. Thank you all.

DECLARATION

I, *Pramoda Niraula*, hereby declare that the work enclosed here is entirely my own, except where states otherwise by reference or acknowledgement, and has not been published or submitted elsewhere, in whole or in part, for the requirement for any other degree or professional qualification. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and listed in the reference section.

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LETTER OF RECOMMENDATION

This is to recommend that the Master's thesis entitled "**Study of Leaf Functional Traits in *Quercus semicarpifolia* and *Quercus lanata* at Altitudinal Gradient in Shivapuri Nagarjuna National Park, Kathmandu, Nepal**" is carried out by Miss. Pramoda Niraula under our supervision. The entire work is based on original scientific investigations and has not been submitted for any other degree in any institutions. We therefore, recommend this thesis work to be accepted for the partial fulfilment of M.Sc. Degree in Botany.

…………………….

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The thesis entitled "**Study of Leaf Functional Traits in *Quercus semicarpifolia* and *Quercus lanata* at Altitudinal Gradient in Shivapuri Nagarjuna National Park, Kathmandu, Nepal**" submitted to Department of Botany, Amrit Campus, Tribhuvan University by "*Pramoda Niraula*", *"5-2-0033-0115-2011"* has been accepted for the partial fulfilment of the requirement for Master's Degree in Botany.

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Pramoda Niraula

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ABSTRACT

The present study shows the leaf traits variation of *Q. semecarpifolia* and *Q. lanata* along the altitudinal gradients and different growing life stages at Shivapuri Nagarjuna National Park. Generalized linear regression model analysis with using the value of R^2 (Coefficient of determination), analyses of variance (ANOVA), mean ± standard deviation was applied for the inter-comparison of the parameters that is, specific leaf area, leaf carbon content , leaf nitrogen content, leaf phosphorus content for testing the differences among the different altitudes, between the species and among various life stages by using SPSS version 25.0 software packages. The statistical analysis shows that there is significance difference in specific leaf area, leaf nitrogen content and leaf phosphorous content along with the altitudes ($p < 0.05$). The result of the study showed leaf carbon content doesn't significantly alters with altitudes ($0.897>0.05$) and also between the species ($0.424>0.05$). Similarly leaf Phosphorous content doesn't significantly alters among the life stage ($0.659>0.05$). A stepwise multiple regression analysis shows a moderate effect of Specific leaf area on carbon ($R^2 = 0.5013$), less effect on nitrogen ($R^2 =0.4277$) and strong effects on phosphorus ($R^2 =0.6708$). Leaf nitrogen and leaf phosphorus was positively correlated with leaf each other and negatively correlated with specific leaf area as the value was less than ($P < 0.05$). Specific leaf area shows no effect on the relationship between carbon, nitrogen, phosphorus and leaf Similarly for stomatal density, the significance value was found to be 0.000 ($p< 0.05$) so there was significance difference in the stomatal density between *Quercus semicarpifolia* and *Quercus lanata* . The density of stomata was found higher in all the plant leaves of *Q. lanata* than *Q. semecarpifolia* irrespective with altitude.

Keywords: Specific leaf area, leaf nitrogen content, leaf carbon content, leaf phosphorus content, Shivapuri, Kathmandu

CHAPTER-1 : INTRODUCTION

Plant functional traits are important tool in plant ecology to understand plant growth, plasticity, species co-existence, and ecosystem-processes (Koerselman *et. al;* 1996). Leaves are the important phenomenon in the life processes of plants as they play a key roles in plant functions and can provide insight in the adaptive evolutionary changes made to suit the surrounding environmental conditions (Guo *et. al.,* 2017). Altitudinal gradient has a major effect on leaf morphology and physiology which provide unique experimental opportunities to study morphological and physiological responses of plants to climate changes (Cordell *et. al.,* 1998).

Plant functional traits are primary drivers of species interaction, community assembly and species diversity that impact individual's fitness indirectly in plant species. Plant communities always have variety of plant species with a wide range of functional traits; therefore, leaf traits are often taken as a means to understand the forest features of the plant communities.

Leaves had a wide range of diversification in their shapes and sizes, number, dry mass, structure and position of stomata, deposition of inorganic (water, carbon-dioxide) substances and organic (proteins, carbohydrates) substances etc. Leaf traits play key roles in plant functions and can provide insight in the adaptive evolutionary changes made to suit the surrounding conditions (Guo *et. al.,* 2017).

Leaves are collectively referred as foliage with many different shapes, sizes, and textures. An immature leaf is defined as a leaf that is newly on the stage of growing for further adaptability on the environment. The immature leaf later turned into the darker green color or tougher texture form so called as mature leaves and ends with the onset of the senescence stage.

Leaf senescence is process of involving remobilization of nutrients from senescing leaves to other parts of the plant. Ethylene is one of the most important hormones in the leaf senescence regulation. During senescence, physiological and biochemical changes are observed including a dramatic metabolic transition from anabolism to catabolism which results in nutrient redistribution to newly developing organs (Lim *et. al.*, 2007).

Elevation and temperature have a strong impact on species composition and leaf traits in mountain systems. Altitude affects the type and amount of sunlight that plants receive, the amount of precipitation received and the nutrients those are available in the soil. Lower elevations tend to have higher temperatures, decreasing in higher elevations (Boscutti *et. al.,* 2018). As a result, certain plants grow very well in high elevations, whereas others can grow only in the mid or lower elevations.

Variations in leaf functional traits (specific leaf area) have guided many studies of functional ecology, which have addressed important ecological correlations, such as relative growth rate and the photosynthetic efficiency of a species (Zhang *et. al.*, 2012). According to (Mallik *et. al*., 2013), of all the environmental factors affecting leaf functional leaf traits, light is one of the most important phenomenon. Plants growing under high light exposure generally show thicker leaves with a lower specific leaf area and shade leaves show a low foliar leaves with less thick lower concentrations of photosynthetic enzymes per area, which increases their specific leaf (Cornelissen *et. al*., 2003, Westoby *et. al*., 2002).

Plants growing in higher elevations are adapted with decreasing height and diameter (Paulsen *et. al*., 2000; Korner 2012). Decrease in diameter growth with increasing elevation must be dominantly caused by the corresponding decline in temperature (Hofgaard, 1997).

In central Himalaya, the plant growth is chiefly associated with the maintenance of a favorable water stress, prolonged dry periods during winter and early summer months. Seasonal fluctuation in rainfall controls the annual cycle of development comprising shoot growth, flowering and leaf fall in individual trees (Borchert 1994 and Poudyal *et. al.,* 2012) and synchronizes tree phenology within forests to some extent. The timing of phenological events can be quite sensitive to environmental conditions. However, differences in precipitation and soil or variations in photoperiod also have major influences on plant phenology(Poudyal, 2017). Deciduous Himalayan leaves complete their expansion late in the dry season, whereas evergreen leaves reach full size during the wet season (Negi and Singh 1992). Leaf fall is seasonal in the Central Himalaya, with 37-53 % of annual leaf fall in the peak month, generally April or May (Singh and Singh, 1987). This concentration of leaf shedding is similar to dry tropical forests but differs from evergreen tropical forests in which leaf fall is relatively consistent throughout the year (Reich, 1995).

Oaks are fundamental to the health of forests, with the highest amount of biomass compared to any other tree species in the forest. Oak forests also make a significant contribution to the quality of the air by carbon sequestration, reducing greenhouse gas emissions, one of the main cause of climate change.

More than 35 species of oaks occur in the Himalayan region, and eight of these species naturally occur in the temperate regions of Nepal (Shrestha, 2003). Naturally, eight species of oak trees can be found in Nepal's subtropical and temperate forests, namely *Quercus floribunda, Quercus glauca, Quercus lamellosa, Quercus lanata, Quercus leucotrichophora, Quercus mespilifolioides, Quercus oxydon,* and *Quercus semecarpifolia*. Oak forests are particularly vital to the populations of hilly regions of Nepal, where local population use oak tree species for fodder, fuelwood, and leaf litter fulfilling a wide range of ecosystem services, such as soil and water conservation, maintenance of sustaining local agriculture in addition to store and sequestrate the atmospheric carbon (Giri and Katzensteiner, 2013).

Leaf traits :

The genus *Quercus* is an evergreen or deciduous tree, belonging to fagaceae family. This genus contain about 450 species and represent an important tree group widespread in Europe, Asia, North Africa, North, Central and South America. *Quercus* species generally grow well in a sunny position, though young plants usually tolerate reasonable levels of shade.

Plants tend to allocate nutrients first at leaves to secure growth as leaf nitrogen and leaf phosphorus are critical for metabolism and are able to use nutrients stored in woody stems to fulfill the needs of leaves when nutrients are limited (Sardans *et. al.,* 2013). The productivity and carbon sequestration of ecosystems depend largely on the availability of nitrogen and phosphorus in plants. Carbon constitutes the basic structure of plants and accounts for 50% of plant biomass. (Wieder *et. al.,* 2015).

Specific leaf area (SLA) is an important trait of plant functional ecology. The most accepted research finding is that species with greater Specific leaf area is more efficient in capturing a limited light resource and SLA reflects the expected return on previously captured resources, and that high-SLA leaves are productive (Klinka *et. al.,* 1992). Low specific leaf area leaves tend to have longer leaf lifespan, presumably because stronger leaf reinforcement is necessary to assure the longer life. Species with high SLA are characterized by high concentrations of nitrogen; high rates of carbon-dioxide and nitrogen uptake per unit leaf and root mass, respectively; and a high rate of photosynthesis per unit leaf nitrogen (Lambers and Poorter,1992). Low specific leaf area species, on the other hand, generally have high values for dry matter content (dry mass: fresh mass); high concentrations of cell walls and secondary compounds; and greater leaf and root longevity. Specific leaf area (SLA) of deciduous species was higher than evergreen species in central Himalayan species (Negi 1989 cited in Singh and Singh 1992). Elevation and canopy shows significant effect on the SLA. For instance,(Luo *et. al*., 2005) shows that, as elevation increased the mean specific leaf area of species and stand decreased from their maximum. Similarly (Chen *et. al.,* 1996) concluded that more shade tolerant species (understories) develop more SLA by decreasing leaf thickness. These understory species when grow in open area increase leaf thickness and decrease specific leaf area. (Klinka *et. al.,* 1992) reported that in general more shade tolerant species have high capacity to change their leaf structure according to environmental conditions.

Specific leaf area (SLA), leaf nitrogen content (LNC), leaf phosphorus content (LPC), and leaf N:P ratio are traits that vary within and among species and are known to be related to plant acquisition and use of resources (Wright *et. al;* 2004). The main bioactive phytochemicals that are found in oak species are phenolic compounds, volatile organic compounds, sterols, aliphatic alcohols and fatty acids. Other compounds that can be found in *Quercus* species are vitamins (especially vitamin E), sterols, aliphatic alcohols and fatty acids (vinha *et. al.,* 2016, . Laemke *et. al.,* 2018).

Low temperature with low Nitrogen (N) and Phosphorus (P) are generally the main limiting factor for plant growth and nutrient supply at high elevations. But global patterns in soil nitrogen and phosphorus limitation or plant nitrogen and phosphorus status have not been well characterized (Sterner, *et. al*., 2002). An increase in foliar nutrient contents occurs with elevation which is interpreted as the plants inability to use the absorbed resources for growth (Petr *et. al*., 2012).

Specific leaf area (SLA) is an important trait of plant functional ecology. In contrast, low-SLA leaves work better in resource-poor environments where retention of captured resources is a higher priority. Low specific area leaves tend to have longer leaf lifespan, presumably because stronger leaf reinforcement is necessary to assure the longer life. Species with high specific area are characterized by high concentrations of nitrogen; high rates of carbon dioxide and nitrogen uptake per unit leaf and root mass, respectively; and a high rate of photosynthesis per unit leaf nitrogen (Lambers and Poorter, 1992). These species are adapted for a high rate of resource acquisition. Low-SLA species, on the other hand, generally have high values for dry matter content (dry mass: fresh mass); high concentrations of cell walls and secondary compounds; and greater leaf and root longevity. These species seem to be adapted for the conservation of acquired resources.

Leaf nitrogen content corresponds to the amount of protein and other secondary compounds present within the leaf, which are necessary for photosynthesis and growth (Wright *et. al.*, 2004). Leaf carbon content per dry mass (LCC) is also a very key factor influencing carbon sequestration in plants. Compared with the nitrogen and phosphorus, Carbon content of plants change slightly, and most studies have accepted the value of 50% however the variations of leaf carbon content among different biomes and life forms remain unclear.(Ma *et. al.*, 2016). Plant nitrogen (N) and phosphorus (P) content regulate productivity and carbon (C) sequestration in terrestrial ecosystems. Phosphorus (P) is more limiting than nitrogen for forest ecosystems being free of disturbances for long periods. Nutrient deficient is part of an organic component or activates an enzyme activity this can be indirectly expressed. For example nitrogen deficiency may be shown by low chlorophyll levels or low activity of nitrate reductive.

As elevation ascends, the air temperature decreases, which is the major environmental factor affecting plant survival, distribution, growth, and reproduction. Common plant adaptive strategies to tolerate low temperature involve minimizing heat loss and the exposure of internal tissues to low temperatures. Thus plants tend to decrease leaf area and increase thickness of leaf, upper and lower epidermis, palisade and spongy mesophyll tissues to increase boundary layer resistance with ascending altitudes (Wang *et. al.*, 2016). Stomata are small pores on the surfaces of plant leaves that act as turgor-operated valves to control the exchange of gases (e.g., water vapor and CO_2) between plant tissues and the atmosphere. Hence, stomata facilitate plant respiration and transpiration and play major roles in the regulation of water and carbon cycling

(Wang *et. al.,* 2014; Wen *et. al*., 2018). In higher elevation with decreased air temperature, CO_2 and O_2 partial pressure, stomatal density has been found to change along these gradients, but patterns are inconsistent (Sun *et. al.,* 2016; Wang *et. al.,* 2014; Yang *et. al.,* 2014). Thicker leaves and epidermis can provide a greater buffer between inner leaf temperature and outer environmental temperature and keep higher internal temperature, which would contribute to maintaining normal physiological activity for plants under low temperatures at higher altitude. In addition, thicker leaves and leaf epidermises can reduce the damage inflicted by the high-level ultraviolet irradiation present at high altitudes (Ma *et. al.,* 2012). It can also facilitate greater water storage and is evolutionarily favorable for efficient water use and reducing transpiration (Guo *et. al.,* 2017).

At higher elevations, harsh environmental conditions and lower resource availability promote stress-tolerant species that invest more carbon on a per-leaf basis As elevation increases, temperature and atmospheric pressure decrease, and clear-sky solar radiation increases, although mountain ranges around the world exhibit different trends of moisture, growing season length and cloudiness (Korner, 2012). Despite the variability in elevation-climate relationships, it may be that at lower elevations, conditions tend to favour acquisitive species that can take advantage of high resource levels, since higher temperature stimulates microbial activity and increases resource availability (Raich and Schlesinger, 1992).

Like elevation, aspect is another major factor in bringing variation in species richness and composition in mountains. Variation in the climatic factors (intensity and duration of light, precipitation, wind velocity and direction etc.), and non climatic factors (slope and nature of terrain, edaphic factors) may also be associated with the aspect of mountains. Though elevation pattern of species richness and composition was explored by most of the researcher in the Himalayan region, very few studies have been attempted so far to explain the influence of aspect that total species richness. A study by (Panthi *et. al;* 2007) has shown that total species richness was found to be significantly higher on the northern aspect than that of south aspect in trans-Himalayan region.

The most frequent relationship between elevation and richness are, in order; a humped shaped species richness curved; a plateau of high species richness with increasing elevation (McCOy, 1990; Rahbek, 1995, McCain, 2005). According to the continuum concept, species composition changes gradually (Whittaker 1956; Austin, 1980), but the change in species composition around the forest-line eco-tone of mountain is rapid. Plant community of a region is a function of time; however, altitude, slope, latitude, aspect, rainfall and humidity play a role in a formation of plant communities and their composition (Kharkwal *et. al*., 2005).In prevailing situation, various phenological changes (flowering, fruiting, germination etc.) in plant communities can be regarded as one of the major influences for various ecological and evolutionary phenomena, including ecosystem productivity, species interactions, community structure, and conservation of biodiversity.

Oaks usually prefer a good deep fertile loam soil which can be on the stiff side. They are also often tolerant to moderate exposure, surviving well but being somewhat stunted seedlings soon develop a taproot and become intolerant of root disturbance. There are more than 35 species of *Quercus* reported from the Himalayan region (Negi and Naithani, 1995), most of which are abundant in temperate forest. Among them , eight species of *Quercus* are mostly reported from Nepal. *Q. floribunda* Lindl., *Q. glauca* Thunb., *Q. lamellosa* Sm., *Q. lanata* Sm., *Q. leuchotrichophora* A. Camus, *Q. mespilifolioides* A. Camus, *Q. oxyodon* Miq. and *Q. semecarpifolia* Sm. n. Major species associated with Khasru in mixed forests are *Q. floribunda*, *Q. lanata*, *Q. leucotrichophora*, *Abies pindrew*, *Rhododendron arboreum*, *Picea smithiana*, *Cotoneaster acuminata*, *Viburnum mullaha*, *Betulautilis*, etc. (DPR, 1997).

Quercus semecarpifolia is a high altitude oak and dominant species of central Himalayan vegetation. It occurs in moist temperate and sub-alpine regions with heavy snowfall and moderate rainfall, and is absent from the dry regions of the inner Himalayas (Negi and Naithani, 1995). *Q. semecarpifolia* is also considered as one of the oldest vegetation of Himalayan zone which invaded the prairieland of the Himalayas and became the dominant species of then sub-alpine and alpine forest (Singh and Singh, 1992).

Quercus lanata is an evergreen tree that can grow up to 30 meters tall. Young shoots are covered in rust- colored hairs. Mature leaves are dark shiny green above and contrastingly rust-colored and wooly-haired beneath, leathery, elliptic-lanceolate, conspicuously toothed, with 9-14 pairs of lateral veins, 8-15cm.

Altitudinal gradient has a major effect on leaf morphology and physiology among the species and also provide unique experimental opportunities to study morphological and physiological responses of plants to climate changes (Cordell *et. al.,* 1998). For example, leaves generally decrease in size and area, while leaf nitrogen concentration and water storage capacity increase with increasing altitude (Korner *et. al.,* 1989; Roderick *et. al.,* 2000). However, negligible studies have been done on leaf traits and their relationships to the distribution of individual species along an altitudinal gradient in Nepal.

Relationship between leaf traits along the altitudinal gradients could not only explain the change in leaf size and area, leaf chemical constituents but also could provide an understanding about the response of plant communities to environmental changes and other mechanisms affecting ecosystem functioning. The presence of leaf-trait relationships along environmental gradients has resulted in the estimation of adaptation of plants to particular environmental conditions.

1.1 Justification :

Quercus semecarpifolia (Khasru) and *Quercus lanata* (Banjh) are two indigenous species of oak, found in the forest of Shivapuri Nagarjuna National Park. *Quercus* species are distributed in wide range of altitude in Shivapuri National Park. Previous studies suggests that altitude has a major effect on leaf morphology, physiology and leaf nutrients. Negligible studies have been done on leaf traits (i.e. phosphorus, carbon, nitrogen, specific leaf area etc.) and their relationships to the distribution of individual species along an altitudinal gradient in Nepal.

Also, there are not much data available about the leaf traits (i.e. phosphorus, carbon, nitrogen, specific leaf area etc.) of these species. Altitudinal gradient has a major effect on leaf morphology and physiology among the species and also provide unique experimental opportunities to study morphological and physiological responses of plants to climate changes. However, negligible studies have been done on leaf traits and their relationships to the distribution of individual species along an altitudinal gradient in Nepal. Shivapuri National Park is very rich in floral diversity due to its location, altitudinal and climatic variations. However, its detailed study is not undertaken till now. Thus, such type of research is expected to fulfill the existing knowledge gap on the status composition of plant species and underlying the factors affecting the forest community in Shivapuri Nagarjuna National Park.

1.2 Hypothesis:

- *Quercus semecarpifolia* and *Quercus lanata* shows leaf traits variation along the altitudinal gradients.

1.3 Research questions:

This research proposal was aimed to investigate the leaf traits in two co-occurring oaks i.e. *Q. semecarpifolia* and *Q. lanata at* Shivapuri, Kathmandu.

1. Does the leaf traits (SLA, leaf carbon, leaf nitrogen, leaf phosphorus) of *Q. semecarpifolia* and *Q. lanata* differ along the altitudinal gradients and different life stages (immature, mature, senescence)?

2. Does the stomatal frequency and stomatal density varies between *Q. semicarpifolia* and *Q. lanat*a along altitudinal gradient at Shivapuri National Park?

1.4 Research Objective:

General Objectives:

- To study the species diversity along altitudinal gradients.
- To study the leaf traits in *Q. semecarpifolia* and *Q. lanata* within different life stages(immature, mature and senescence) along the altitudinal gradient.

The specific objective of this research is:

- Enumeration of associated species to know the status of diversity and vegetation composition.
- To study the different leaf parameters such as; specific leaf area (SLA), leaf carbon content(LCC), leaf nitrogen content (LNC), leaf phosphorus content (LPC) during different life stages in both species, *Q. semecarpifolia* and *Q. lanata*.
- To study the stomatal density of *Q. semecarpifolia* and *Q. lanata* species.

CHAPTER-2 : LITREATURE REVIEW

Plant species of the changing habitats of higher elevation are the sensitive indicator of global climate change, which showed increased growth rates in recent years (Motta and Nola, 2001). The species diversity will increase or decrease with increasing elevation depends on interaction among plant communities, species and environmental factor (Brown, 2001). The variation in species richness is caused by many components of climate and local environment (e.g. temperature, precipitation etc.) that vary along the elevation gradients and ultimately create the variation in species richness (Lomolino, 2001).

Grytness and Vetaas (2002) analysed plant species richness along the Himalayan altitudinal gradient in Nepal. They concluded that interpolated species richness in the Himalaya showed a hump shaped structure. The maximum richness of flowering plants of Nepal has been found between 1500 m- 2500 m a.s.l. Total species richness from lowland to 6000 m a.s.l. in Nepal indicated very little variation between 3000 m to 4000 m a.s.l. and in general, species richness remains fairly constant generating a high-elevation plateau. A decreasing trend in species richness with increasing altitude is reported by several workers (Odland and Birks, 1999). However, others reported a hump shaped relationship between species richness and altitude (Baniya *et. al*., 2012).

Forest ecosystem plays a significant role in global carbon cycle which store large quantities of carbon in vegetation soil and exchange with the atmosphere through photosynthesis and respiration (Dixon *et. al.,* 1994). Carbon storage in forest ecosystem is strongly affected by climate, forest type, stand age, distribution and edaphic condition (Pregitzer and Baskirchan, 2004). On regional and continental scale, the forest biomass and soil survey show that the carbon density of forest biomass and soil affect by the role of climate and vegetation type with spatial pattern (Dixon *et. al.,*1994). The vegetation carbon density decreases but soil carbon density increased with increasing latitude. The carbon distribution in temperature and boreal forest affect by the stand age and distribution regimes (Gower, *et. al.,* 1997). Many environmental factor (e.g. temperature, precipitation, atmospheric pressure, light, wind) change with altitude.

Kearney *et. al.,* 2006, defined Leaf functional trait analysis is a useful tool in plant ecology and has played an important role in theories related to plant growth, plasticity, species co existence, and ecosystem-level. Experiments and field measurements on vegetation have revealed a number of apparently universal trends in the co-variation of key leaf properties across a wide range of environments.

Most of the research concludes that tree-line species shows increased population density and growth rates under global change. Decreased basal area but increased seedling and sapling density of tree with increasing elevation indicates the better adaptation of the species under improved climate (Hofgaard 1997). In central Himalaya (Saxena and Singh, 1984) analyzed the size class distribution and population structure of major dominant species such as ,*Quercus* spp., *Acer oblongum, Rhododendron arboreum* etc., which show mixed type of population structure and regeneration. Temperature seems to be the limiting factor controlling the population and growth of most plant species more than other environmental factors in higher elevations (Korner, 2012). Study on some palatable species such as *Quercus* spp. Shrestha (2003) showed that they are easy to regenerate by plantation and can only be managed by sustainable looping removing of old and dying trees to make the canopy more open.

The most striking trend is the so-called global spectrum of leaf economics (Reich *et. al.,* 1997, Wright *et. al*., 2004, 2005). Vetaas (1977) studied the effect of canopy disturbance on species richness in a central Himalayan Oak forest and reported that Phanerophytes did not respond to any variable. Yadav and Sah (1998) studied quantative analysis of vegetation (trees and shrubs) in Nagarjuna hill. They found *Schima wallichii* and *Castanopsis tribuloides* as the most dominant species of Nagarjuna hill and observed that the highest density of *Castanopsis tribuloides* showed their maximum adaptability on north-east facing slopes which are relatively undisturbed forest of Nagarjuna hills with a result that farther the sampling plots along the altitudinal gradient, lesser was the similarity between the present plant species.

Study of variations in leaf functional traits, especially in specific leaf area (SLA), have addressed important ecological correlations, such as relative growth rate and the photosynthetic efficiency of a species by many researchers (Reich *et. al.,* 2003; Zhang *et. al.,* 2012). Elevation is a complex environmental gradient, associated globally with declining atmospheric pressure and temperature, whereas abiotic factors such as solar radiation or precipitation show more regional patterns (Korner, 2003, 2007). Shade leaves show a low foliar construction cost, since they are less thick and with lower concentrations of photosynthetic enzymes per area, which increases their specific leaf area (Westoby *et. al.,* 2002).

Sigdel (2003) studied altitudinal coordinated pattern of plant community structure in different altitudinal ranges of Shivapuri National Park and found that the pattern of distribution of plant species was not uniform according to altitude. The genotypes growing in the colder, more seasonal environments at high elevation generally produce shorter plants with thicker leaves and more tolerant of frost than at lower elevations (Korner, 2003; Vitasse *et. al.,* 2014) while plants growing under high light exposure generally show thicker leaves with a lower SLA (Cornelissen *et. al.,* 2003).

The forest had abundant number of small seedlings but sapling was very rare and the survival of large seedling and sapling appear to limit the regeneration of *Quercus semecarpifolia* (khasru) forest in Shivapuri Hill (Shivapuri National Park, Kathmandu) Shrestha *et. al.,* (2004). Wright *et. al.,* (2004) showed that the investments of plants in structural and chemical leaf traits have a return in terms of physiological activity. The spectrum goes from plant species that have a high return in physiological activity (i.e. high leaf nutrient content and high photosynthetic rate) to species with a lower potential rate of return (i.e. low leaf nutrient content, low photosynthetic rate). In terms of leaf morphology, the high return rate is related to less tough leaves (i.e. low leaf mass area, leaf thickness (LT) and leaf tissue density (LTD)) while the opposite is true for leaves characterized by a low return strategy.

Environmental factors, such as air temperature, radiation, and soil nutrients, may vary with altitude (Korner, 2007; Soethe *et. al.,* 2008). The interplay between temperature and photoperiod (i.e. day-length) affects phenological processes such as flowering time, budburst, seasonal stem growth, leaf senescence and dormancy (Rossi *et. al.,* 2006, Luo *et. al.,* 2018, Tylewicz *et. al.,* 2018).

Patterns of plant functional trait variation inform not only on community structure but also on population dynamics and the mechanisms affecting ecosystem functioning. Shifts in plant functional traits within or between sites and/or species across environmental gradients may also reflect deterministic processes of community organization (Silva and Batalha, 2009). Elevation gradients therefore offer an opportunity to study adaptive traits under strong selective pressure to overcome the homogenizing effect of gene flow (Gonzalo-Turpin and Hazard, 2009; Sexton *et. al.,* 2011). At higher elevation, the forest was mature with almost closed canopy and trees were large; so the tree density was low.

Despite the apparent ubiquity of variation in plant functional traits with elevation, and the growing literature documenting patterns along single elevation gradients, global-scale analyses are needed to show repeated elevation gradients in form and function within and among species across systems that may point to universal underlying mechanisms (Poorter *et. al.,* 2009). Circumstantial evidence points towards a potentially important day-length effect also on stomata conductance (Zhao *et. al.,* 2009). The increase in light availability in disturbed forests understories, as a consequence of the lower canopy height and gaps, causes physiological and morphological responses to the understory species which might involve a reduction in their SLA (Ishii and Asano, 2010).

Carbon is relatively more stable than mineral elements in plants and can be easily measured simultaneously with other key elements (Han *et. al.,* 2011). Thus the ratio of carbon(C), nitrogen (N), phosphorus (P) in plants is widely used in 38 ecological stoichiometry to diagnose nutrition limitation, competition, and biogeochemical cycle (Sardans *et. al.,* 2012; Liu and Sun, 2013). Of all the parameters of leaf functional traits, light is perhaps one of the most studied in the literature (Pearcy, 2007; Hulshof and Swenson, 2010; Mallik *et. al.,* 2013). Despite the apparent ubiquity of variation in plant functional traits with elevation, and the growing literature documenting patterns along single elevation gradients, global-scale analyses are needed to show repeated elevation gradients in form and function within and among species across systems that may point to universal underlying 4mechanisms (Poorter *et. al;* 2009; Korner 2012).

Quercus semecarpifolia, Smith. (brown oak) forests dominate the high altitudes of Central Himalaya and these forests have large reserves of carbon in their biomass (above and below ground parts) and soil (Verma *et. al.,* 2012). The nutritive value of the leaves of *Q. semecarpifolia* was continually changing, especially carbon, phosphorus and soluble protein. *Q. semecarpifolia* harvested at the proper stage of maturity (winter months) offers considerable potential as high quality forage for livestock to fulfill the deficiency of protein (Singh *et. al.,* 2012). For instance, temperature will decrease, while precipitation and radiation will increase with a rise in altitude (Read *et. al.,* 2013; Li *et. al.,* 2017; Wang *et. al.,* 2017). Such variations might influence leaf traits.

Conversely, at higher elevations, harsh environmental conditions and lower resource availability promote stress-tolerant species that invest more carbon on a per-leaf basis (Korner *et. al.,* 1989; Korner 2012). Day-length has also been documented to be a driver of seasonal changes in the photosynthetic capacity of leaves and ecosystems at similar or even larger importance as temperature (Bauerle *et. al.,* 2012**).**

Awareness of local-scale variation in many leaf traits for individual species, as well as the relationships among these traits and their dependence on altitude, might be essential for extrapolating eco-physiological processes from the leaf to the ecosystem level (Navas *et. al.,* 2010). The inclusion of within-species trait variability in community assembly analyses substantially improves the detection of ecological patterns and processes (Jung *et. al;* 2010). A decrease in species diversity at high versus low-elevations can also be associated with a reduction in species interactions, which would lead to a relaxation of plant defenses at high elevation (Rasmann, *et. al.,* 2014).

Elevation-related patterns of stomata characteristics in leaves are primarily a function of plant functional trait can reveal the adaptation strategies of plants, and help us to predict their responses to future climate changes (Wang *et. al.,* 2014). Long photoperiods are considered as indicators of "time to grow" and declining photoperiods as indicators of "time to prepare for winter" (Korner *et. al.,* 2016). Altitudinal gradients can be used to study and analyze the adaptive features of terrestrial plants under the influence of global climate change (Li *et. al.,* 2017; Wang *et. al.,* 2017). Vapor pressure deficit is a major driver of seasonal changes in transpiration, but photoperiod also modulates leaf responses while climate warming and day-length is a significant driver of the seasonal variation in stomata conductance (Elena *et. al.,* 2019). Hence, the importance of functional leaf traits as a guide to understanding forest changes, this thesis was aimed to study the leaf traits of two co-occurring oaks, *Q. semecarpifolia* and *Q. lanata* at Shivapuri, Kathmandu.

CHAPTER-3 : MATERIALS AND METHODS

3.1 Study Area:

Shivapuri Nagarjuna National Park is located in the country's mid-hills on the northern fringe of the Kathmandu valley. It covers an area of 159 km^2 in the districts of Kathmandu Nuwakot and Sindhupalchowk. SNNP has some important pilgrimage destinations including Nagi Gumba, Bagdwar, Bishnudwar, Jamacho as well as Manichur Mahadev, Kageshwori, Sundarimai, Baudeshwor, Tarkeshow, Pachali Bhairav etc. Bishnumati, Nagmati originated from Shivapuri Nagarjuna National Park are main sources of drinking water for Kathmandu Valley. Total Elevation of the park area ranges between 1350 m to 2732 m at Shivapuri peak. However, most of the park area lies between 1,600 m and 2,500 m above sea level (SHNP Management Plan 2004).

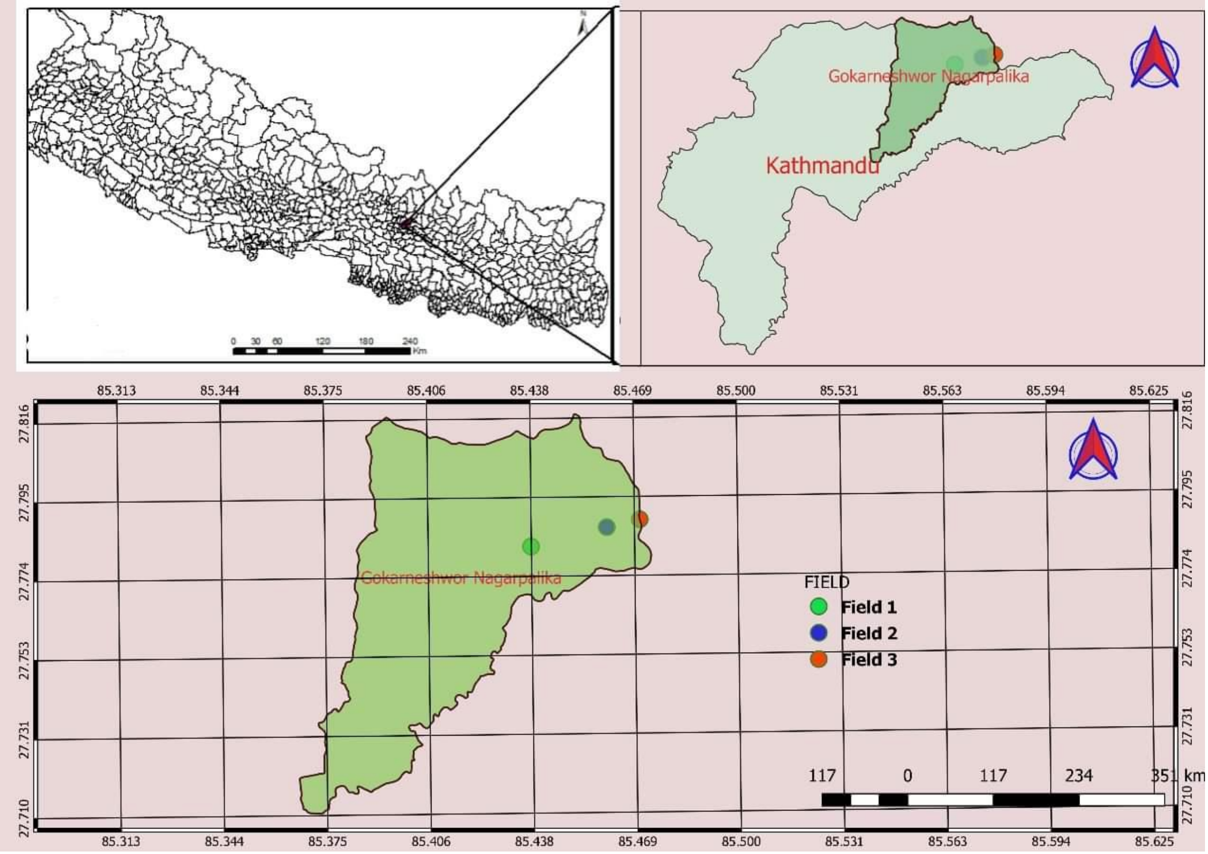

Figure 1. Study area map with sampling locations in Shivapuri Nagarjuna National park

Geographially, Shivapuri, is located between 27°45" to 27°52" N and 85°16" to 85°45"E and Nagarjuna is located 27°43" to 27°46" N and 85°13" to 85°18"E. The area was gazetted as the national park in 2002. Initially it was established as Shivapuri Watershed Reserve in 1976, Shivapuri Watershed and Wildlife Reserve in 1984 later. It was declared as the ninth National Park in the country under the National Park and Wildlife Conservation Act 1973 and the National Park and Wildlife Conservation Regulation 1974. It adopted the IUCN Management Category II of Protected area, Covering an area of about 144 km^2 of Kathmandu, Nuwakot and Sindhupalchowk districts of Central Development Region.

3.2 Vegetation:

Shivapuri Nagarjuna National Park lies in a transition between subtropical and temperate climates. The park represent mid-hill ecosystem of Nepal. It is famous for globally threatened wildlife, birds, and butterflies. It is also a good site for forestry, park management, ecology etc. It support rich flora and fauna diversity with the number of protected, threatened and endemic species of mammals, birds and plants. It harbors a total of 1250 plant species including rhododendron, pine, and oak etc. with 16 endemic flowering plants i.e. *Aechmanthera claudiae*, *Pteracanthus rotundifolius*, *Impatiens insignis*, *Impatiens leptoceras*, *Begonia flagellaris*, *Maharanga wallichiana*, *Silenepseudo cashmeriana*, *Eriocaulonkath manduense*, *Homalium napaulense*, *Ormosia glauca*, *Rota larubra*, *Rubus hibiscifolius*, *Pedicularis wallichii*, *Carum diversifolium*, *Peucedanum nepalense*, *Pleurospermum rotundatum* (KMTNC, 2004).

Nepal Biodiversity Resource Book (2007) has recognized Evergreen-mixed broadleaved forest (1000m-1500m) that includes *Schima-Castanopsis* forest (dominated by *Schima wallichii* and *Castanopsis indica*), Chir Pine and Broad-leaved forest (1000m-1600m) (dominated by *Pinus roxburghii, Alnus nepalensis,* 17 *Rhododendron arboreum, Symplocos pyrifolia*), East Himalayan Oak-Laurel forest (1500m-2700m) dominated by *Quercus glauca, Q. lamellosa, Q. lanata*, *Lindera pulcherrima,*) and Temperate Mountain Oak forest (2300m-2700m) dominated by *Quercus semecarpifolia*) forest. SNNP listed 2122 species of flowering plants including 16 endemic species. However, recent study documented 1254 species including 132 pteridophyte species, 6 gymnosperms, 325 monocotyledons and 791 dicotyledons.

The common vegetation include *Schima wallichii*, *Caszzztanopsis indica*, *Alnus nepalensis*, *Pinus roxburghii*, *Myrica esculanta*, *Pyrus pasia*, *Quercus semecarpifolia*, *Quercus lanuginose*, *Rhododendron arboreum*, *Juglans regia*, *Taxus wallichiana* etc. (Bagmati river basin improvement project, 2013).

The forest of Shivapuri National Park is dominated by *Q. lanata* at lower elevations and *Q. semecarpifolia* (khasru) at higher elevations. Regeneration of khasru seems very poor in comparison to *Q. lanata*. A preliminary study showed that khasru forest had only old dying trees and seedlings but no individuals between these two size classes (Shrestha and Lekhak 2002), a clear indication of inadequate regeneration. The regeneration is continuous in the nearly undisturbed forest of khasru in Langtang National Park, Central Nepal (Vetaas, 2000) but such a situation was not observed in SNP (Shrestha and Lekhak 2002) indicating that absolute conservation does not ensure continuous regeneration of this species. The forest shows prominent signs of decline. Abnormal growth and branching (i.e., clusters of thin, profusely branched and slender branches with shorter internodes), increased defoliation and dying back of leader and branch tips, which are frequently observed in the forest, are sure signs of decline (Larcher, 1995).

According to Singh (2014), 428 species of vascular plants belonging to 112 families and 323 genera were documented from the Shivapuri National Park. Among them pteridophytes comprised of 50 species, Gymnosperms 3 species and Angiosperms 375 species (Dicotyledons 285 species and Monocotyledons 90 species). Floristic analysis 24 showed that Dicotyledons comprise 65.9%, Monocotyledons 21.5% , Gymnosperms 0.7% and Pteridophytes 11.9%. In the present study the ten largest families having higher number of species are: Compositae (34 specie), Gramineae (33 species), Leguminosae (17 species), Orchidaceae (16 species), Labiatae (14 species), Rosaceae (14 species), Rubiaceae (13 species), Cyperaceae (12 species), Polygonaceae (10 species), and Zingiberaceae (9 species).

Shivapuri Nagarjuna Park provides an ideal place for scientific research , regarding climate change, watershed management, biodiversity, linkages of forests, hydrological cycle as the valley is undergoing catastrophic environmental degradation primarily from air pollution, respiratory related illness caused from burning of fossils fuel, leaves , a thick cloud of pollutants, mainly carbon-dioxide suspended in the air for a long period until wind or rain disperses and also helpful in sequestering carbon from the source and reducing the time taken to clean the valley's environment (DPR, 2015).

3.3 Climate:

Shivapuri has subtropical to warm temperate type of climate. There is a high variation in annual temperature and precipitation. The maximum temperature recorded in the year 2010 ranged from 25^o C -28 ^{o} C and the minimum temperature range recorded was 10^o C-10.5^o C in 2012 . Annual rainfall was maximum in the year 2013 with 1800mm and minimum rainfall was recorded in the year 2017 (1000 mm). In 2019, the year of this study, maximum temperature was 26^o C (June) and the minimum temperature recorded was 19^o C (December). (Department of Hydrology and Meteorology).

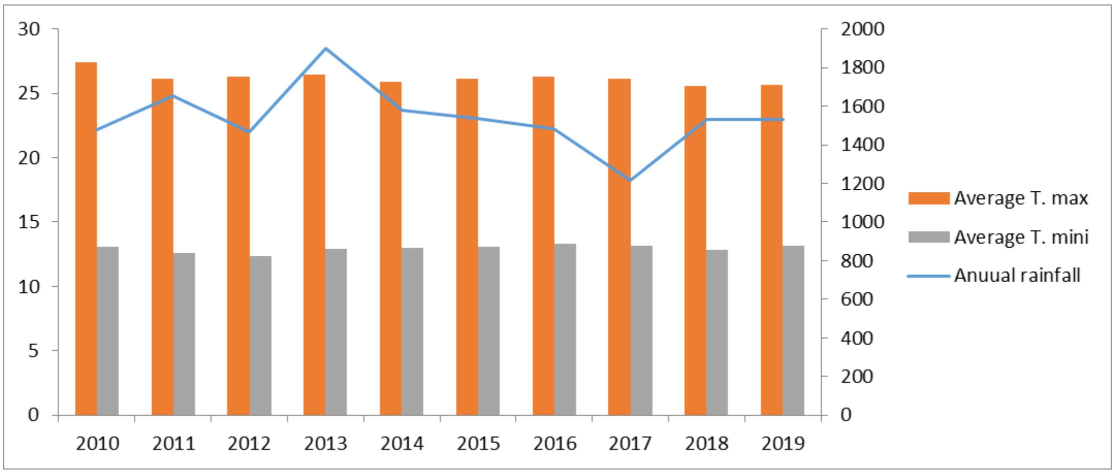

Figure 2: Average Temperature and Precipitation of Kathmandu Station of Nine Years (2010-2019).

(Source: Department of Hydrology and Meteorology / Government of Nepal, Kathmandu).

3.4 Soil Properties:

The soil of Shivapuri Nagarjuna National Park is under different categories as given below: overused soil (1122ha), soil-needing treatment (10,090 ha), soil used within capability (6,491 ha), serious used soil (9491 ha) and under use soil (2,350 ha) (ICIMOD, 2004). The region has seasonal climate with rainy (monsoon) season (June to September), cool dry winter (October to February) and hot dry summer (March to May). Soils of the study area contain metamorphic rocks such as phyllite, limestone and dolomite, gneiss and ingratiate which are loamy on the northern aspect and sandy on the southern (Shrestha, 2003).

Due to the dense vegetation covers, in most of the park area, the run off rate is relatively low and the nutrient content in the soils is high. In contrast, in the degraded forest areas the rate of run-off and soil erosion is relatively high. The soils of the area range from loamy sand on the northern side to sandy loam on the southern slope (SHNP Management Plan, 2004). Due to the dense vegetation covers, in most of the park area, the runoff rate is relatively low and the nutrient content in the soils is high.

3.5 Methodology:

3.5.1 Site and Leaf Selection:

Three different altitudes i.e. 1632m (Site A), 2020m (Site B), and 2414m (Site C) were selected. In each altitudinal range, quadrat was applied for leaf sample collection of *Quercus semecarpifolia* and *Quercus lanata* species. The main criterion for defining leaf status was leaf stickiness. If one rubbed a finger over a mature leaf, the waxy coating made the leaf feel slick. One could apply pressure, and the finger would slide across the surface without causing damage. Such leaves are found close to the apical meristem of new flush . Immature leaves have a lighter green color but even fully expanded leaves are still light green. A finger rubbed across the surface will tend to stick (slightly), and with pressure, the leaf will roll and tear. Immature leaves were light green, not fully expanded, of variable age. Mature leaves were dark green, fully expanded. Immature leaves were easily identified by a light touch, or subtle changes in color. Leaf yellowing due to chlorophyll degradation is often considered to be the main marker for leaf senescence. Only undamaged leaves were used in experiments.

3.5.2 Leaf Collection:

Two hundred and seventy circular plots of 10m radii were laid in each three altitudes i.e. 1632m(Site A), 2020m(Site B) and 2414m(Site C) by using quadrat for leaf sample collection. Leaf sample collection was carried out in three different months i.e. May, August and December. New leaves appear in May and leaf fall begins during the same period, but most of the new leaves attain full size before the completion of leaf fall. Sometimes, the maturity of leaves takes place from July to August, while leaf senescence starts from the month of November, and the evergreen trees stands leafless for a brief period. However, leaf fall and new leaf emerges varies with altitude, aspect and micro-climate. Foliar phenology of Khasru in central Nepal (Shivapuri National Park) is different from the pattern mentioned above.

Shrestha and Lekhak (2002) reported completely leafless trees during early September. Fifteen leaves from healthy plants of each species that lie within the quadrat were taken to study the leaf parameters, stomata observation and were further taken for lab work. Leaf collection was done for three times considering three different life stages (pre-mature, mature and senescence).

3.5.3 Vegetation Sampling:

Vegetation sampling was done using circular plots of radius 10m radii for trees at three different altitudinal gradient i.e. 1632m(Site A), 2020m(Site B) and 2414m(Site C). In each sample plot, the woody plant species (only tree) were recorded along with following structural attributes (DBH greater than 10cm , height greater than 20cm). All dominant species and associated species in each plot was observed and recorded. Most of the plant species were identified during field survey. Local name of the plants was identified with consultation of local people. Plant samples were made and photographs were taken for unidentified species in the field. These were then identified by consultation with experts and using books by Polunin and Stainton (1984), Stainton (1988) and Ghimire, *et.* al., (2008) as reference. Local, English and Scientific name and their proper citation were made with the help of books by Shrestha (1998). The diversity indices at three different altitudinal gradient i.e. (1632m, 2020m, 2414m) was observed for vegetation analysis.

3.5.4 Data management and Vegetation analysis:

Vegetation analysis was carried out by using Zobel *et. al.,* 2000 using following formulas:

Density and Relative Density (RD)

Density is the numerical strength of a species where the total number of individuals of each species in all the quadrats is divided by the product of total number of quadrats studied and area of single quadrat. Density is calculated by the equation:

$$\textbf{Density} = \frac{\textbf{Total no.of species occured}}{\textbf{Total no.of quadrat studied}} \times \frac{\textbf{1}}{\textbf{area of quadrat}}$$

Relative density is the study of numerical strength of a species in relation to a total number of individuals of all the species and it is calculated as:

$$\textbf{Relative Density} = \frac{\textbf{Density of individual species}}{\textbf{Total density of all species}} \times 100\%$$

Frequency and Relative Frequency (RF)

Frequency deals about degree of dispersion of individual species in an area and usually expressed in terms of percentage and can be calculated as:

$$\textbf{Frequency} = \frac{\textbf{No.of quadrat in which species occured}}{\textbf{Total no.of quadrat studied}} \text{ x } 100\%$$

Relative frequency is the degree of dispersion of individual species in relation to the dispersion of all the species occurred and calculated as:

- $\textbf{Relative Frequency} = \frac{\textbf{Frequency of individual species}}{\textbf{Total frequency of all species}} \text{ x } 100\%$

Basal Area And Relative Basal Area:

Basal area is the common term used to describe the average amount of an area . It is defined as the total cross-sectional area of all stems in a stand measured at breast height, and expressed as per unit of land area (typically square feet per acre).

- $\textbf{Basal Area} = \textbf{Total Basal Area of Species x } \frac{\textbf{10,000}}{\textbf{Size of the plot}}$
- $\textbf{Relative Basal Area} = \frac{\textbf{Basal area of individual species}}{\textbf{Total basal area of all species}} \textbf{ x 100}$
- **Above ground carbon stock (AGCS) = Dry wt. of leaf x 0.47**

Importance Value Index (IVI):

Importance value index deals that which plant species is more important and dominant (i.e. in terms of relative density, relative frequency and relative density) in the study area and calculated by using formula given by (Zobel *et al.*, 1987).

- Importance Value Index (IVI) = Relative Frequency (RF) + Relative Density (RD) + Relative Coverage (RC)

Species Diversity Indices:

Diversity measures take account of two factors, viz: species richness i.e. number of species present and evenness i.e. the similarity of the population size of each of the species present. For the analysis of vegetation following diversity indices were calculated.

Simpson's Diversity Index (D)

It is calculated by using 1-Simpson's Index, as the value of D increases then species diversity also increases and its value ranges from 0-1.

- Simpson’s Index (D) = $\frac{\Sigma\, n(n-1)}{N(N-1)}$

Shannon Diversity Index (H)

It is derived by Shanon and Weaver (1945) independently. It is a measure of diversity that combines species richness (the number of species in a given area) and their relative abundances. It is calculated using formula:

$$H = \sum P_i \ln P_i$$

$$\text{Evenness} = \frac{\text{Shanon diversity index (H)}}{\text{Maximum Possible Value of H (Hmax)}}$$

$$H_{max} = \text{Ln (species richness)}$$

Where, Pi = Proportion of individual species

3.6 Statistical analysis:

The significance of differences of both the species *Quercus semecarpifolia* and *Quercus lanata* between sites were evaluated by using Statistical Package for Social Sciences (SPSS, version 16.0.0) i.e. for ANOVA, correlation and regression test. Linear regression was done using SPSS to find level of significance as well as relationship between altitude and species diversity. Species diversity along altitude was compared in two ways; firstly, Species number present within 100m interval were recorded along altitude. Linear regression was done between species richness and altitude as scatter plot, for this data sheet was made up of number of species present in each quadrat along with respective altitude. Most of the graphical representation was made using MS-Excel.

3.7 Study of Stomatal structure, density :

A fresh replicate of five healthy leaves from both the species of *Quercus semecarpifolia* and *Quercus lanata* from three altitudes, 1632m, 2020m, 2414m (Site A, Site B, Site C) was taken from each three altitudes for stomata observation. Then, transparent imprint of 1cm^2 was prepared from a healthy leaf of average size by applying nail varnish on the abaxial surface at both sides of the main vein to a 1cm^2 patch on the leaf abaxial surface to make a transparent imprint of the leaf surface. After drying, the nail varnish in the leaves of both *Quercus semecarpifolia* and *Quercus lanata* leaves, the nail varnish was removed with pincers, and mounted it on a microscope slide for stomata observation.

Stomatal density (SD) was estimated by counting the number of stomata from 3 different altitudinal fields by using ocular scale micrometer of view on each leaf sample at 40 X 15 magnification. From each of these images, the guard cell length and breadth of five randomly selected stomata were taken. Therefore, for each species, the stomatal density was calculated and the length of guard cells was measured.

3.8 Estimation of leaf nutrients:

The leaf parameters (specific leaf area, leaf phosphorus content, leaf nitrogen content, leaf carbon content) were estimated from the selected (Site A, B, C) altitudes in the field at Shivapuri. Fifteen fresh leaves of both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* were collected from three selected different altitudes and kept on a air tight zipper bag and brought on a ASCOL campus lab for observation . Then, the length and breadth of the leaves from both the species were taken and were kept on drying oven for drying leaves. The dry weight of leaf was taken after drying in an oven at 60°c for 72 hours to calculate specific leaf area. The specific leaf area (cm^2 /g) was calculated as a relation of leaf area (LA) / dry weight of leaf.

Furthermore, for estimation of leaf nutrients, dried leaf samples was then grinded to a powder form. The powdered sample of leaves was taken to lab of Central Department of Environment, Tribhuvan University. Estimation and calculation of leaf nutrients was performed using following methods.

3.8.1 Leaf Nitrogen Content (LNC):

Leaves nitrogen was estimated by micro-Kjeldahl method (Karla, 1998) which proceeded in three steps: Digestion, Distillation and Titration.

Digestion: 0.2 gram of dry and sieved leaves, 2 gm. of digestion mixture and 10ml of conc. H_2SO_4 were taken in the digestion flask and the sample was digested in low heat until the frothing was stopped. Temperature was raised about 400°c taking care that the acid condenses at about one third the way up the neck of digestion flask. The flask was swirl at intervals till the carbonaceous particle was present and the color changes to greenish blue. The digested mixture was cooled to room temperature and about 40 ml distilled water was added before the solution starts crystallizing. The solution was transferred in a 100ml volumetric flask washing the digestion flask with 3-4 lots of small amount to the mixture with gentle shaking. Then 20 ml. of 4% boric acid in 125 ml. Erlenmeyer flask was taken and 4 drops of mixed indicator was added and was placed under the condenser.

Distillation: The Kjeldahl distillation flask with digested materials was assembled on distillation chamber. 20 ml. of 40% sodium hydroxide solution was poured down the neck holding the flask at 45° angle so that it turns to the bottom of the flask without mixing. Now the flask was attached quickly to the distillation unit and swirl to mix. Now the distillation flask was heated to boiling but avoid sucking back of boric acid. Distillation was continued till the distillate is about 75 ml. Again the nitrogen was determined by titrating the distillate with 0.05 Hcl. The color of the mixed indicator just changes from blue to reddish at the end point.

Calculation :

$$\% \ N = \frac{(S - B) \times n \times 14 \times 100 \times 100}{W \times 1000 \times 20} = \frac{(S - B) \times n \times 7}{W}$$

Where,

S = volume of standard acid (ml) used up by sample

B = volume of standard acid (ml) used up by blank

n = Normality of the standard acid.

W = oven dry weight of sample

14 = equivalent weight of nitrogen

20 = Aliquot

3.8.2 Leaf Phosphorus Content (LPC):

Available leaves phosphorus was estimated by Vnadomolybdo- Phosphoric system method. Besides simplicity, it has greater tolerance to the interfering elements than any other methods. The acid concentration in the determination is not so critical however final concentration of 0.3N to 0.8N acidity is recommended. The sensitivity varies along 10 fold between the wave lengths 400 and 400µu with the higher sensitivity at the low wavelength. Therefore, for the determination of yellow colour of phosphorus , the 420µu wavelength is recommended.

Dry ash extract: 5 ml aliquot of extract solution was taken in 50 ml volumetric flask and was diluted to 35 ml. Now 10 ml of vanadomolybdate reagent and was diluted to 50 ml. with distilled water and was mixed. After that, the yellow color was measured after 20 min. at 420mu and as compared with that of the phosphorus standards.

Calculation :

Ppm P in plant = ppm P in solution x 10W

Where,

W = oven dry weight of plant sample and 5 ml aliquot is taken from 50 ml dry ashing extract.

% P in plant = ppm P in plant x 100/ 10^6

Wet digestion extract :10 ml. aliquot of the wet digestion extract was pipetted in 100 ml. of beaker and evaporate to dryness. The residue was dissolved in 5 ml. of 2N HNO3 warming if necessary. Now it was transferredto 25 ml. volumetric flask with distilled water and make up the volume. 10 ml. aliquot of the distilled solution was taken in the 50 ml. volumetric flask and proceed to determine phosphorus by vanadomolybdate phosphoric yellow methods as dry ashing extract.

Ppm P in plant = ppm P in solution x W

Where,

W = oven dry weight of plant sample and 10 ml aliquot is being used to control the acidity and 10 ml. aliquot was taken for colour development.

% P in plant = ppm P in plant x 100/ 10^6

3.8.3 Leaf carbon content (LCC):

Leaf carbon is an important plant trait and also a very key factor influencing our estimations of regional and global carbon sequestration in plants. Compared with the nitrogen and phosphorus, Carbon content of plants change slight, and most studies have accepted the value of 50%, however, the variations of leaf carbon content among different biomes and life forms remain unclear (Ma *et. al*; 2016). For leaf carbon, the dry weight of leaf was taken after drying in an oven at 60°c for 72 hours. The leaf area content was calculated as a relation of dry weight of leaf x 0.47%.

CHAPTER-4 : RESULTS

4.1 Results:

4.2 Important Value Index (IVI):

The Important Value Index provides a quantitative basis for the classification of community in three 1632m(SiteA), 2020m(Site B), 2414m(Site C) along the altitudinal gradient at Shivapuri Nagarjuna National Park.

In 1632m (Site A) altitude, the highest IVI was recorded for *Quercus lanata* ,while at 2020m (Site B) altitude the highest IVI was recorded for *Quercus semecarpifolia* followed by altitude 2414m (Site C)(Fig.3). Similarly, the lowest IVI was recorded for *Rhododendron arboreum*.

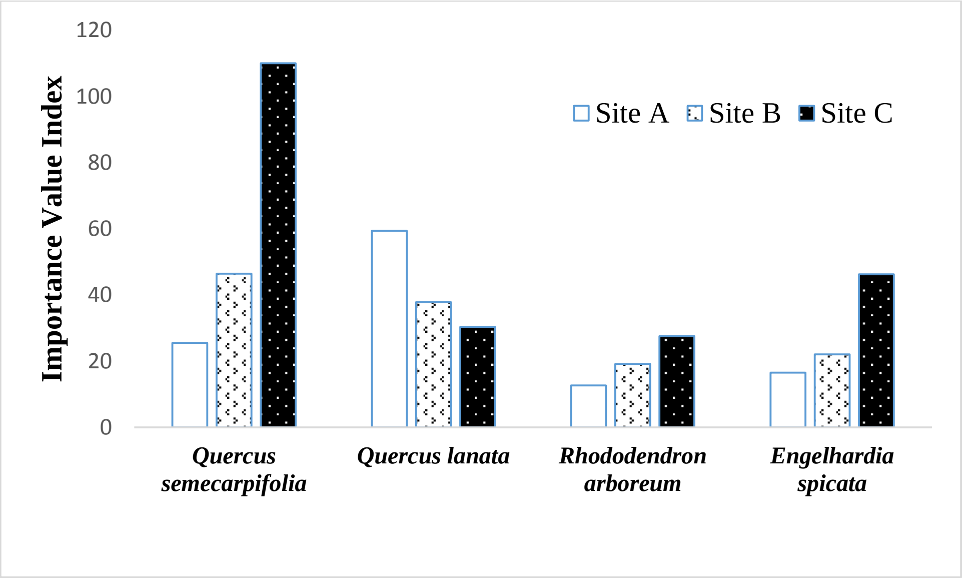

Fig. 3 : Importance Value Index for the studied plots in the Shivapuri forest

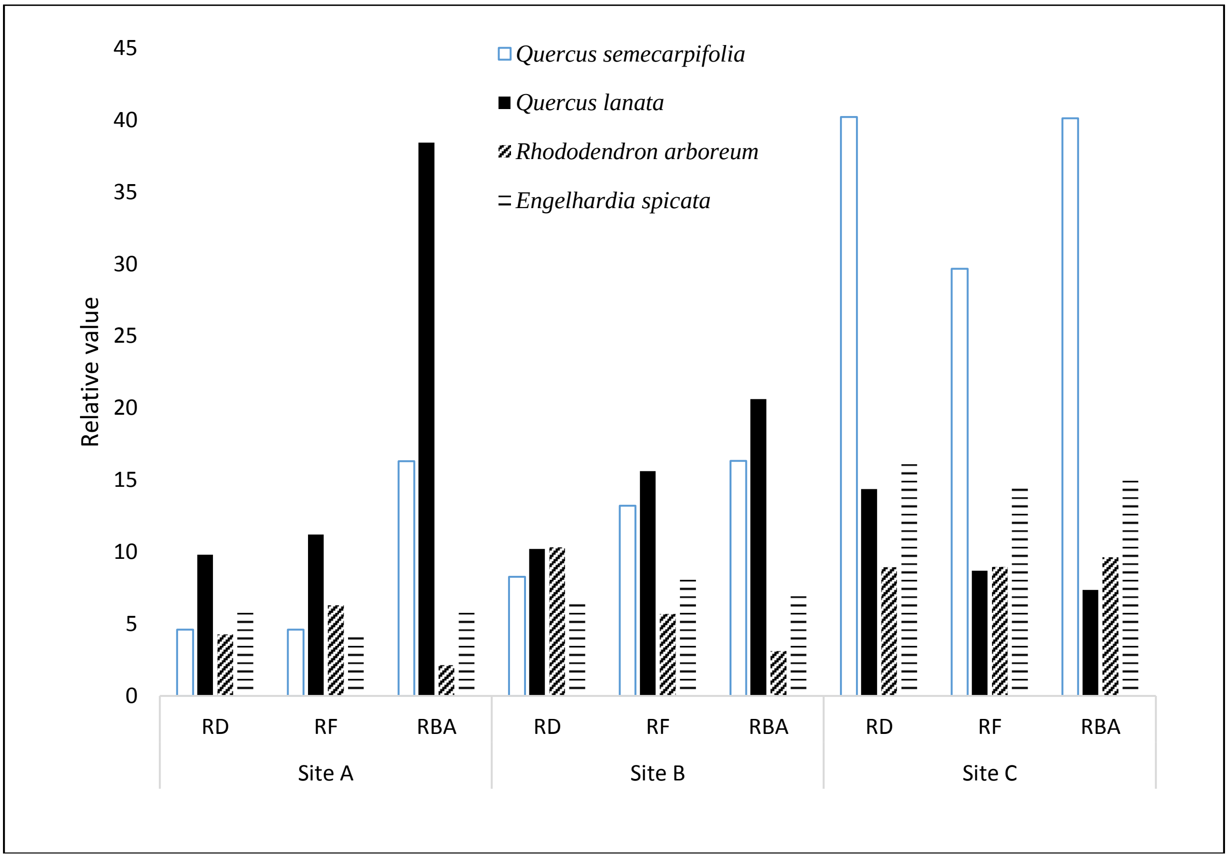

Fig. 4 Graph showing relative percentage for the studied plots in the Shivapuri forest at Site A,B, C

Quercus lanata showed high Relative density, Relative frequency, Relative basal area at 1632m (Site A) and 2020m (Site B) and *Quercus Semecarpifolia* showed highest number of relative data value at 2414 (Site C) (Fig. 4).

The total number of tree species (species richness) was highest in site C(Table-1). The major associated tree species were *Alnus nepalensis, Schima wallichii, Lyonia ovalifolia* etc. at lower altitude, *Castanopsis tribuloides, Quercus glauca, Symplocus ramosissima, Myrsine capitellata* at middle altitude and *Quercus lamellosa,* and *Eurya acuminata* at higher altitude. At mid elevation 2020m, *Rhododendron arboreum* and *Quercus lanata* were the most frequent and dominant species. At the higher altitude *Quercus semecarpifolia* was the most dominant species. *Pinus roxburghii* was the ecologically most important tree species in higher altitude. The value of Simpson index and Shanon index showed species richness and evenness was high at 2020m and 2414m altitude. *Quercus* forest showed high number of species as well as high canopy cover including major tress species like *Symplocos* sp., *Lyonia* sp., *Eurya* sp.

Simpsons index for tree species was 0.09 and Shanon Wiener index(H) of species diversity was 2.44 while the evenness was 0.82 for Site A. Similarly, Simpsons index for tree species was 0.11 and Shanon Wiener index(H) of species diversity was 2.40 while the evenness was 0.90 for Site B. Simpsons index for tree species was 0.11 and Shannon Wiener index(H) of species diversity was 2.40 while the evenness was 0.88 for Site C.

Table 1: Species diversity index of tree species along altitudinal gradient

	SITE A (1632m)	SITE B (2020m)	SITE C (2414m)
Simpson's Index	0.09	0.11	0.11
Shannon Wiener Index (H)	2.44	2.40	2.40
Evenness	0.82	0.90	0.88

4.3 Specific Leaf Area:

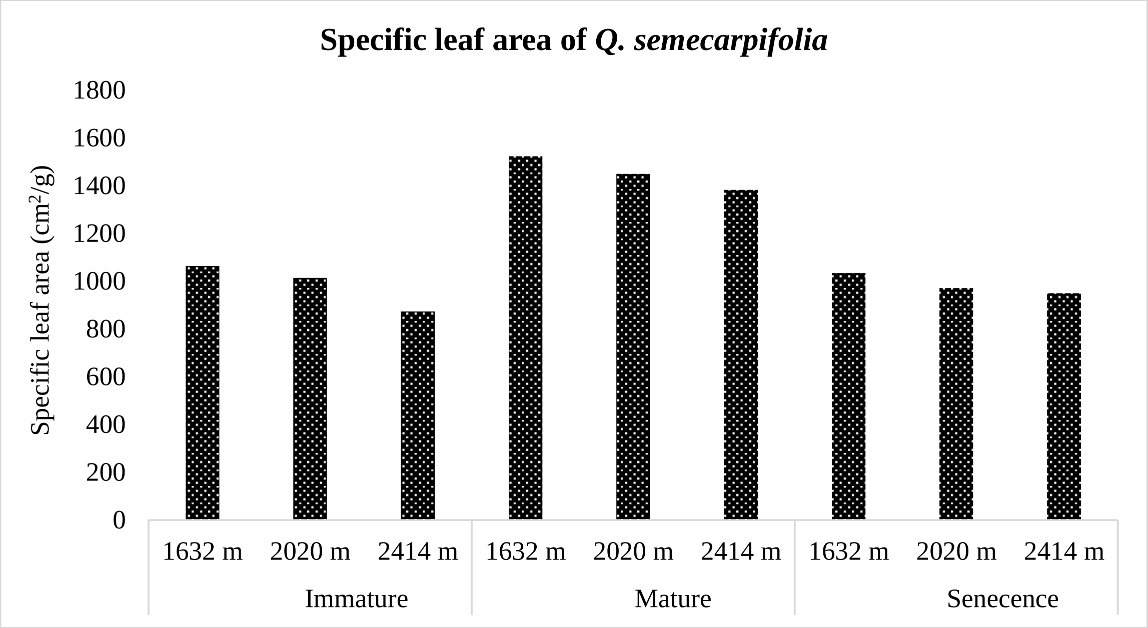

Fig. 5 Graph showing specific leaf area of *Q. semecarpifolia* along altitudinal gradient and life stages

Specific leaf area of *Quercus semicarpifolia* (khasru) leaf was found highest in mature stage at site A .The lowest specific leaf area was found in immature leaf at Site C. The specific leaf area was found increasing at Site A and was found slowly decreasing along with the increasing altitudinal gradient.

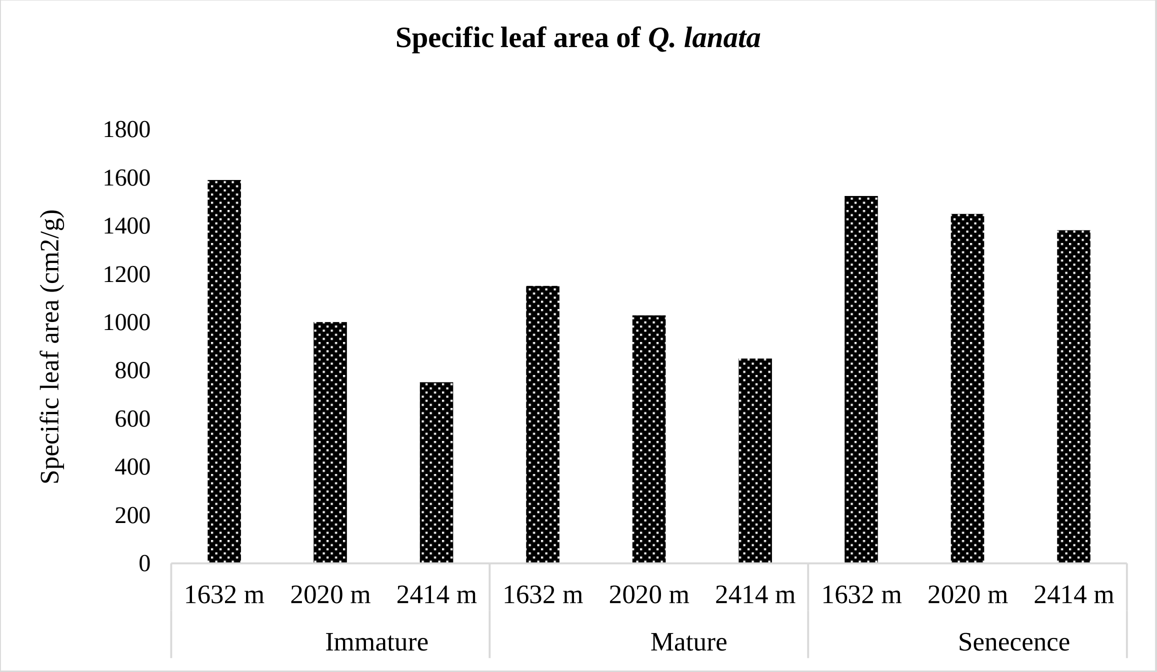

Fig. 6 Graph showing specific leaf area of *Q. lanata* along altitudinal gradient and life stages

Specific leaf area of *Quercus lanata* was found highest in immature leaf at 1632m altitude (Site A). The lowest specific leaf area was found in immature leaf at 2414m altitude (site C) almost similar to mature leaf.

4.4 Leaf Carbon Content:

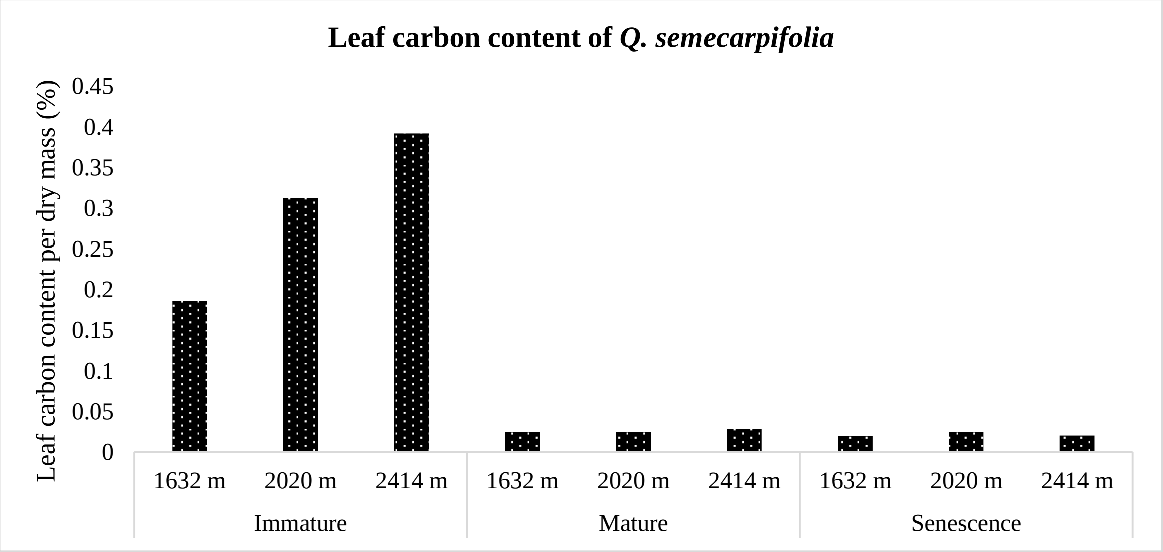

Fig. 7 Graph showing leaf carbon content of *Q. semecarpifolia* along altitudinal gradient and life stages

Leaf carbon content of *Quercus semicarpifolia* was found highest in immature leaf at 2414m altitude (Site C). The lowest carbon content was found in mature and senescence leaf at 1632m altitude (site A) followed by 2020m (Site C) altitude.

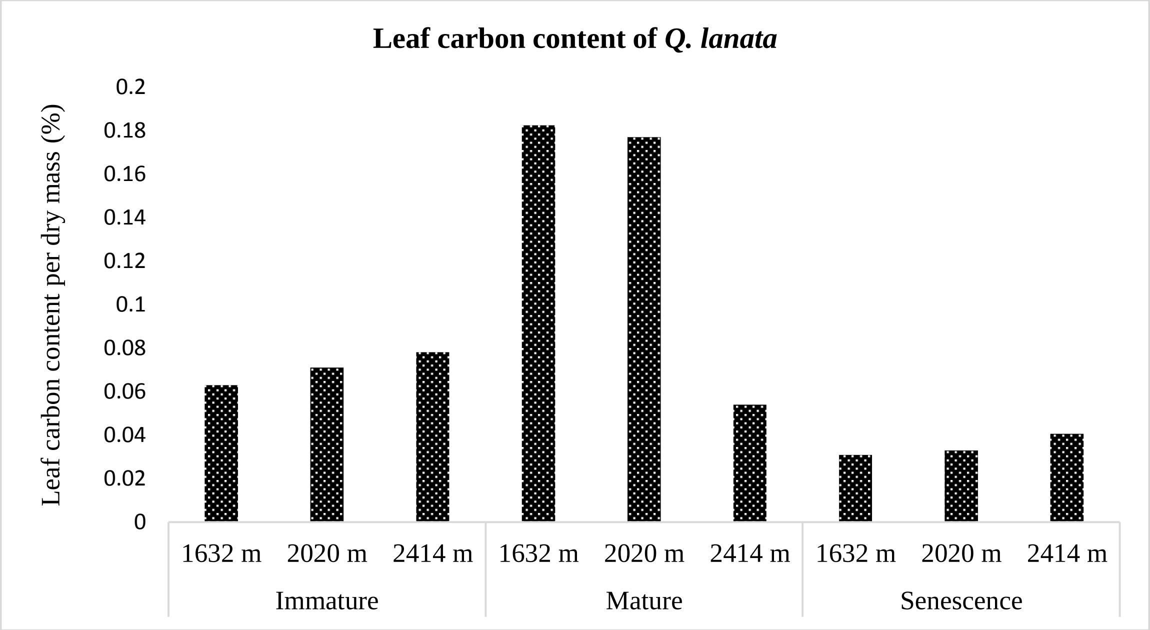

Fig. 8 Graph showing leaf carbon content of *Q. lanata* along altitudinal gradient and life stages

Leaf carbon content of *Quercus lanata* was found highest in mature leaf at 1632m altitude (Site A) and the lowest was observed at 1632m altitude (site A) of senescence leaf similar to 2020m (Site C) altitude.

4.5 Leaf Nitrogen Content:

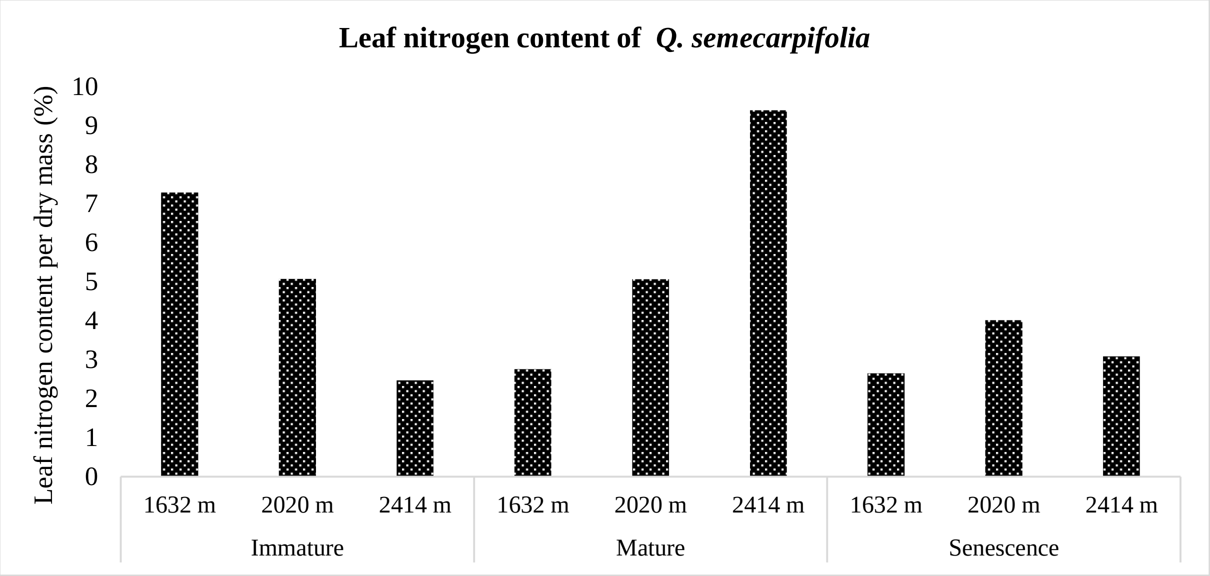

Fig. 9 Graph showing leaf nitrogen content of *Q. semecarpifolia* along altitudinal gradient and life stages

Leaf nitrogen content of *Quercus Semicarpifolia* in leaf was found to be highest in immature leaf at 1632m (site A) altitude. The lowest was at 2414m altitude (site C) followed by 1632m (Site A) altitude of senescence leaf.

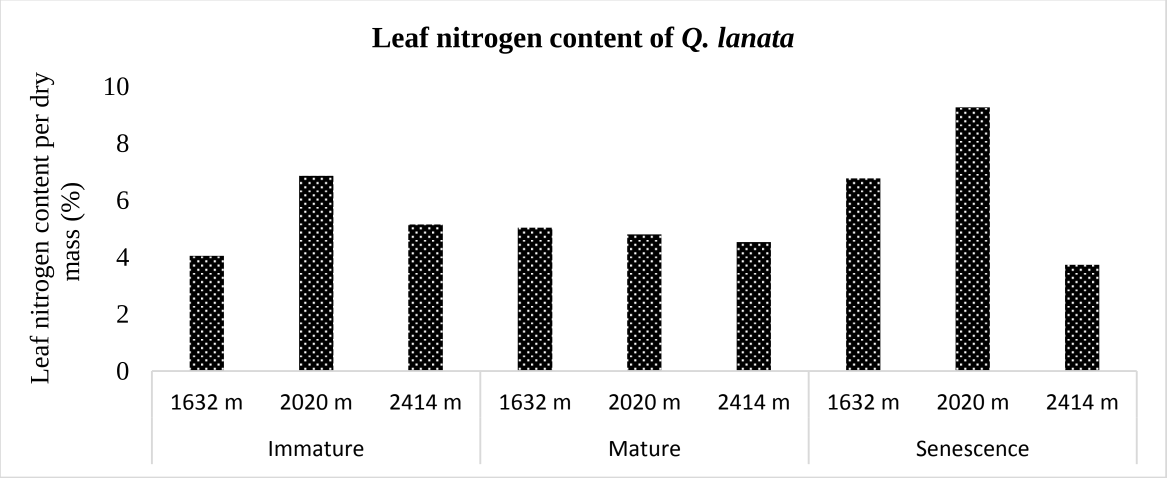

Fig: 10 Graph showing leaf nitrogen content of *Q. lanata* along altitudinal gradient and life stages

Leaf Nitrogen Content of *Quercus lanata* was found to be highest in immature leaf at 1632m altitude (Site A). The lowest was at 2020m altitude (site B).

4.6 Leaf Phosphorous Content:

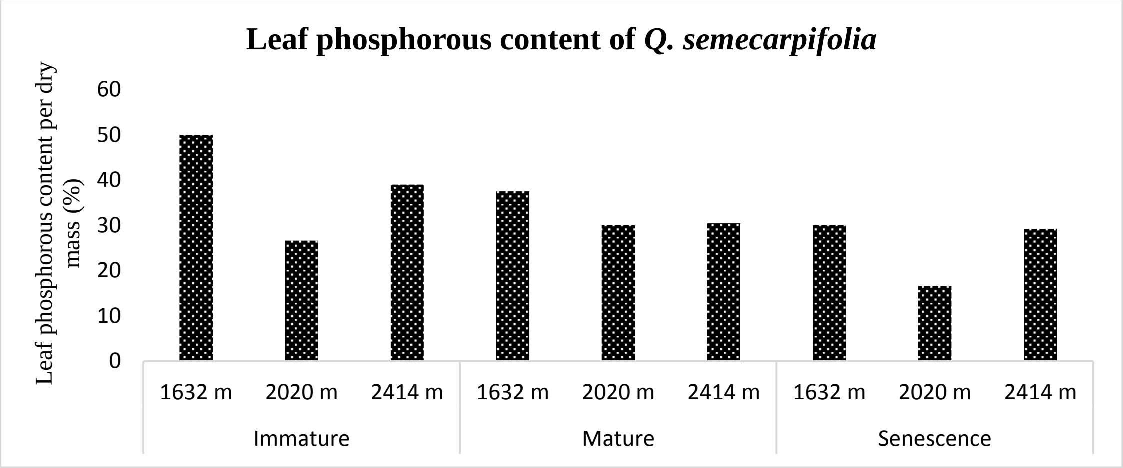

Fig. 11 Graph showing leaf phosphorous content of *Q. semecarpifolia* along altitudinal gradient and life stages

Phosphorus content of *Quercus semicarpifolia* in leaf was found highest in immature leaf at 1632m altitude (Site A). The lowest phosphorus content was found in senescence leaf at 2020m altitude (site B).

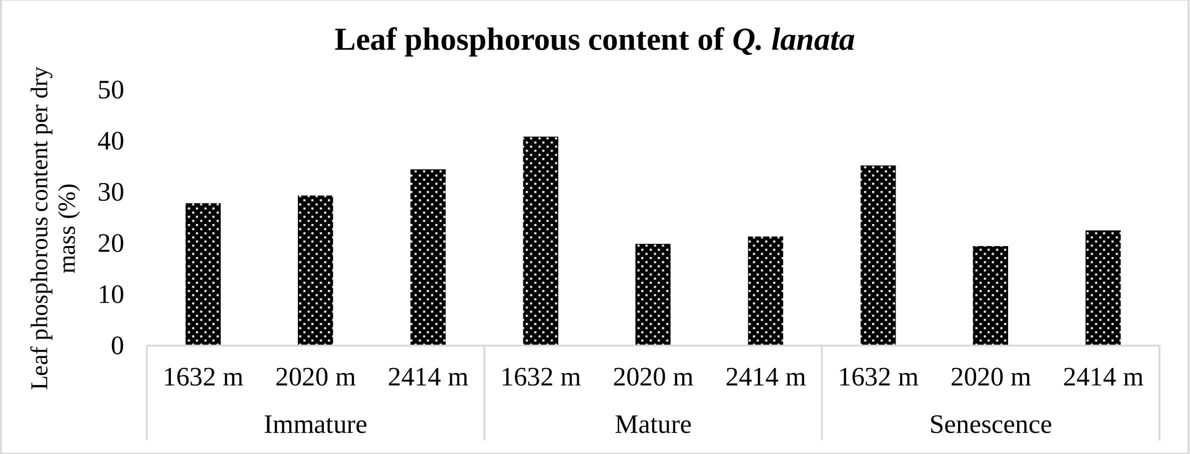

Fig. 12 Graph showing leaf phosphorous content of *Q. lanata* along altitudinal gradient and life stages

Phosphorus content of *Quercus lanata* species was found highest in mature leaf at 1632m(Site A) altitude. Phosphorus content was found almost similar in immature and senescence leaf according to the test performed. The lowest phosphorus content of *Quercus lanata* was found at 2020m altitude (Site B).

4.7 ANNOVA test:

The statistical analysis shows that there is significance difference in specific leaf area along with the altitudes and life stages(i.e. immature, mature and senescence) between both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* as the significance value was found 0.05>0.033. It doesnot show any significance difference if we observed individually both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* along the altitudes (1632m, 2414m, 2024m) and life-stages (i.e. immature, mature and senescence) as the significance value was found more than 0.05<0.148, 0.05<0.080(Table-2). (Annex-I)

Table -2: ANOVA test of specific leaf area between *Q. semecarpifolia* and *Q. lanata*

Source	**df**	**Mean Square**	**F-value**	**p-value**
Altitudes	2	51764488.63	1.92	0.148
Life stage	2	68368300.50	2.54	0.080
Species	1	50261130.28	1.86	0.173
Altitudes *Lifestage	4	71413252.41	2.65	0.033
Altitudes * Species	2	81364009.38	3.02	0.050
LifeStage * Species	2	82318313.72	3.05	0.048
Altitudes *LifeStage * Species	4	85138822.53	3.16	0.014

The statistical analysis shows that leaf carbon shows significance difference in different life stages(i.e. immature, mature and senescence) between both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* as the significance value was found 0.05>0.003. But it does not show any significance difference along the altitudinal gradient (1632m, 2020m, 2414m) by both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* as the significance value was found more than 0.05<0.894. Also the data shows there is no any significant difference in between the altitudes and life stages as the value was 0.05<0.733 (Table -3)(ANNEX-2).

Table -3: ANOVA test of leaf carbon content between *Q. semecarpifolia* and *Q. lanata*

Source	**Df**	**Mean Square**	**F-value**	**P-value**
Altitudes	2	0.01	0.11	0.894
LifeStage	2	0.75	4.80	0.009

Species	1	0.10	0.65	0.418
Altitudes *LifeStage	4	0.07	0.50	0.733
Altitudes *Species	2	0.08	0.53	0.588
LifeStage * Species	2	0.90	5.80	0.003
Altitudes *LifeStage * Species	4	0.03	0.21	0.931

The statistical analysis shows that leaf nitrogen shows significance difference in different life stages (i.e. immature, mature and senescence) and/between along the altitudinal gradient (1632m, 2020m, 2414m) by both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* as the significance value was found less than 0.05<0.000, 0.05<0.000, 0.05<0.003, 0.05<0.013(Table-4)(ANNEX-3).

Table -4: ANOVA Test of Leaf Nitrogen Between *Q. semecarpifolia* And *Q. lanata*

Source	df	Mean Square	F-value	P-value
Altitudes	2	3754.17	61.85	0.000
LifeStage	2	1492.07	24.58	0.000
Species	1	743.33	12.24	0.001
Altitudes *LifeStage	4	845.48	13.93	0.000
Altitudes * Species	2	349.65	5.76	0.003
LifeStage * Species	2	268.07	4.41	0.013
Altitudes *LifeStage * Species	4	500.75	8.25	0.000

The statistical analysis shows that leaf phosphorus shows significance difference in different life stages (i.e. immature, mature and senescence) and/between along the altitudinal gradient (1632m, 2020m, 2414m) by both the species i.e. *Quercus semecarpifolia* and *Quercus lanata* as the significance value was found less than 0.05<0.000, 0.05<0.000, 0.05<0.003, 0.05<0.013. But individually shows no significant differences in life stages (i.e. immature, mature and senescence) as the given value was more than 0.05 i.e. 0.05<0.483(Table-5) (ANNEX-4).

Table -5: ANOVA test of leaf phosphorus between *Q. semecarpifolia* and *Q. lanata*

Source	df	Mean Square	F-value	P-value
Altitudes	2	48.34	9.28	0.000
LifeStage	2	3.79	0.72	0.483
Species	1	78.90	15.14	0.000
Altitudes *LifeStage	4	104.33	20.03	0.000
Altitudes * Species	2	58.01	11.13	0.000
LifeStage * Species	2	143.76	27.60	0.000
Altitudes *LifeStage * Species	4	116.29	22.33	0.000

4.7 Regression Analysis:

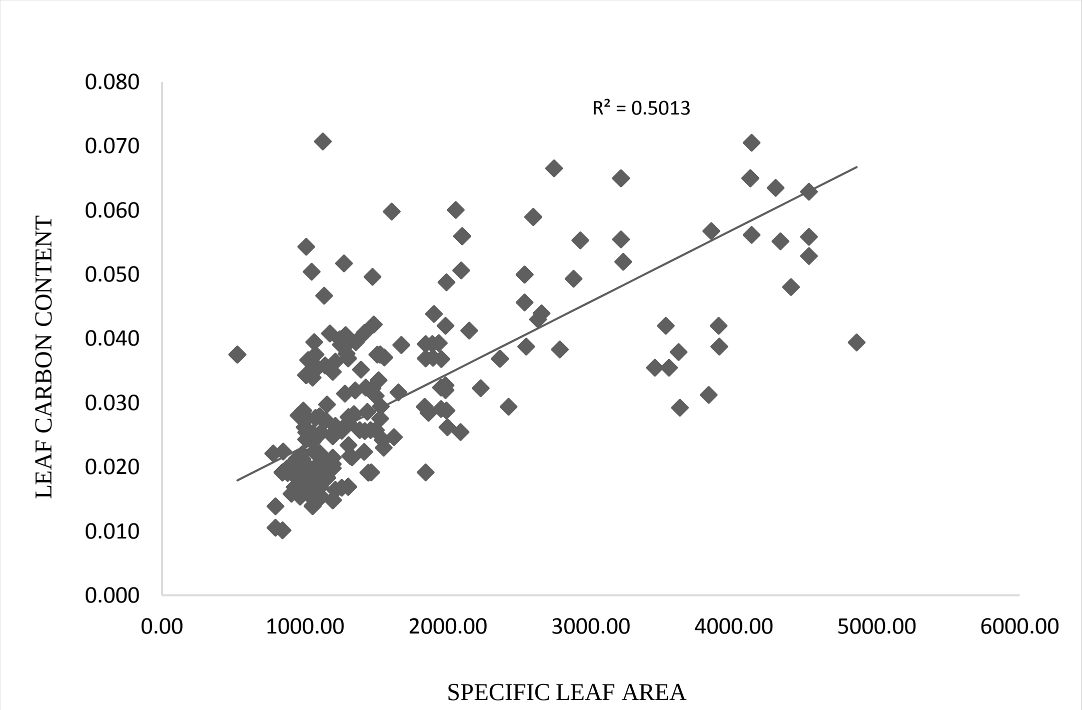

Fig. 13: Linear regression plot between specific leaf area and leaf carbon content

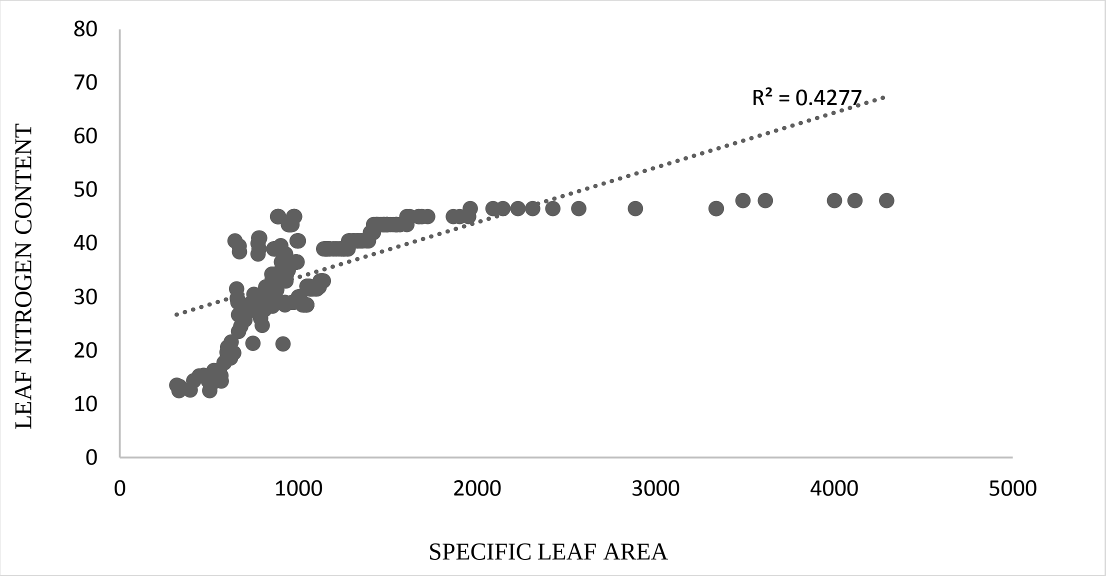

Fig.14 Linear regression plot between specific leaf area and leaf nitrogen content

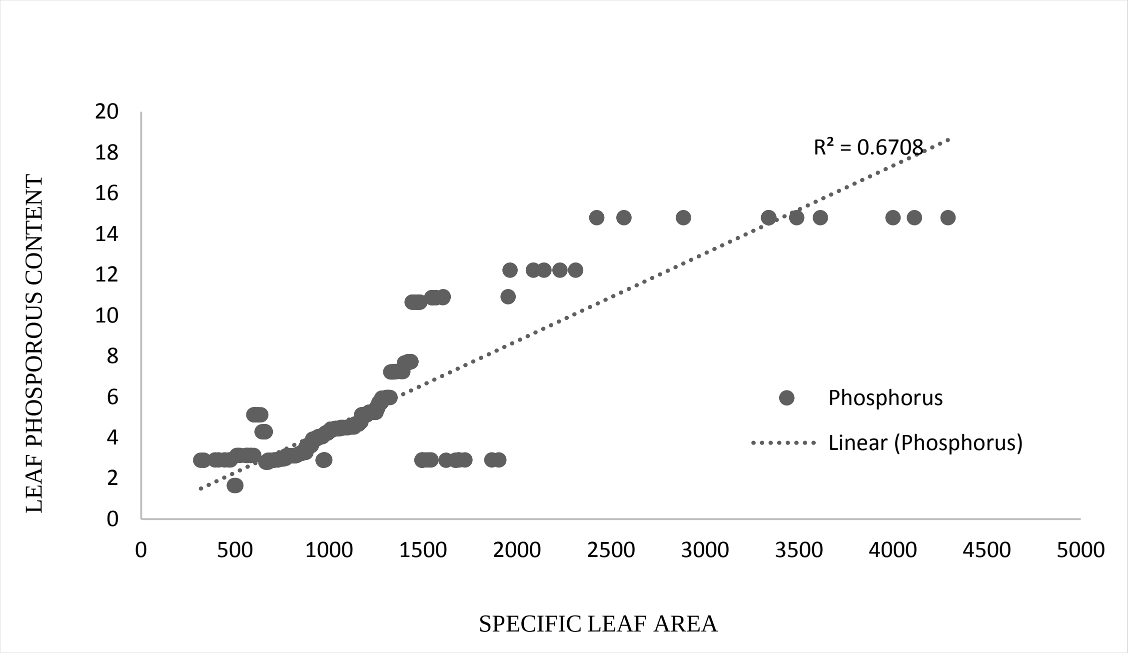

Fig. 15 Linear regression plot between specific leaf area and leaf phosphorous content

A stepwise multiple regression analysis shows a moderate effect of Specific leaf area on carbon($R^2 = 0.5013$). Less effect on nitrogen ($R^2 = 0.4277$) and strong effects on phosphorus ($R^2 = 0.6708$). Leaf nitrogen and leaf phosphorus was positively correlated with leaf each other and negatively correlated with specific leaf area as the value was less than ($P < 0.05$). Specific leaf area shows no effect on the relationship between carbon, nitrogen, phosphorus and leaf area.

Changes in the nitrogen and phosphorus concentration were small, indicating that the plant nutritional status did not, necessarily, change very much on the altitudinal gradient. There were, nevertheless, large changes in nitrogen and phosphorus, mainly due to the changes in the specific leaf area, which could affect the functioning of the photosynthetic system. Our study indicates that a coordinated plant response modified these parameters simultaneously across different altitudes.

4.8 Stomata Analysis:

Table 6: ANOVA test of stomatal density between *Q. semecarpifolia* and *Q. lanata*

	Sum of Squares	Df	Mean Square	F	Sig.
Between two species	318843.66	1	318843.66	67.02	0.000
Within Groups	133193.99	28	4756.92		
Total	452037.65	29			

The significance value was found to be 0.000 (p value less than 0.05). Therefore, the statistical analysis shows there is significance difference in the stomatal density between *Quercus semicarpifolia* and *Quercus lanata* along the altitudinal gradient at Shivapuri .

Table 7: Table showing stomatal density of *Q. semecarpifolia* and *Q. lanata*

S.N.	stomatal density of *Quercus semecarpifolia*	stomatal density of *Quercus lanata*
1	251.44	402.31
2	226.30	452.60
3	201.15	502.89
4	226.30	477.74
5	150.86	402.31
6	150.86	377.16
7	301.73	452.60
8	226.30	301.73
9	201.15	251.44
10	176.01	553.18
11	201.15	477.74
12	176.01	427.45
13	226.30	502.89
14	352.02	477.74
15	301.73	402.31
Mean	224.62	430.81
Standard deviation	57.39	76.86
Standard error	14.81	20.36

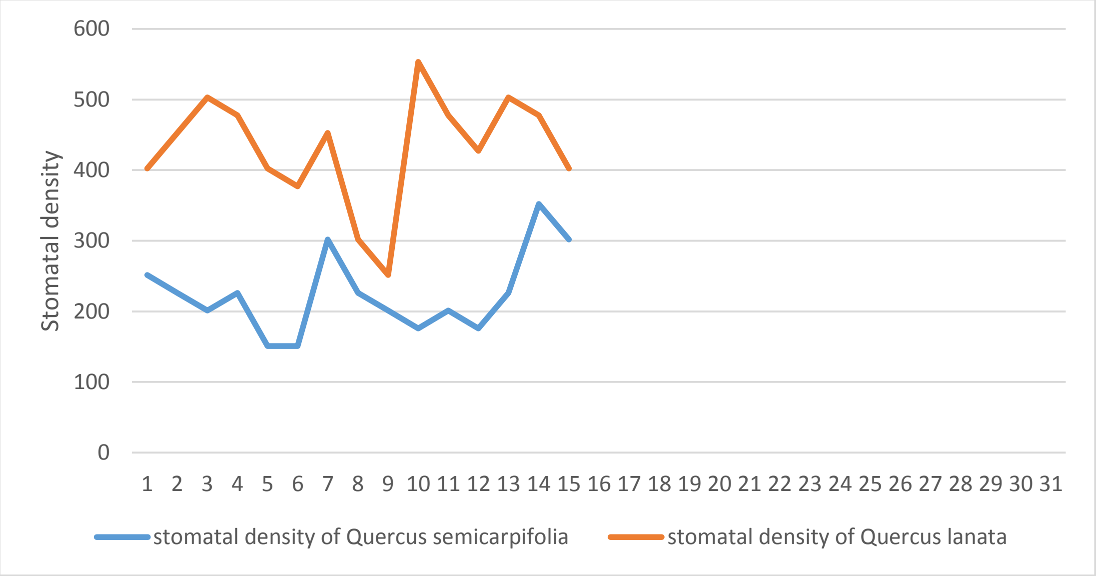

Fig. 16 Graph showing stomatal density of *Q. semecarpifolia* and *Q. lanata*

4.9 Density of stomata:

There was a marked variation in the density of stomata in the plant leaves of *Q. semecarpifolia* and *Q. lanata* (Table 6, 7). The density of stomata was found higher in all the plant leaves of *Q. lanata* than *Q. semecarpifolia* irrespective with altitude.

CHAPTER- 5 DISCUSSION AND CONCLUSION

5. Discussion:

Functional traits are predictors of plants in response to environmental stimuli. They represent specific functional adaptations to various environmental stresses. This study deals with the variation in plant functional traits along altitudinal gradient.

Leaves are the most important component of a plant's photosynthetic apparatus and play a vital role in plant function and long-term adaptation to environmental change (Liu *et. al;* 2016). Specific leaf area is trait that have been widely used to predict growth strategy and the response to environmental variations. Therefore, studying leaf functional traits is important for scaling up to predict community or ecosystem-level functions and processes (Scheepens *et. al;* 2010, Daneshgar *et. al;* 2012). In mountain habitats, there can be large variations in temperature, moisture, light, soil properties, and other abiotic factors along altitudinal gradients (Read *et. al.,* 2014; Li *et. al;* 2018; Wang *et. al;* 2018). All these changes can greatly affect leaf functional traits and ultimately may result in some obvious changes to plant growth and development as well as population expansion (Dong, 2011).

Variation in leaf traits also influence geographic patterns in nitrogen and phosphorus. If climate variation alters leaf life-span or leaf mass per area, these changes could affect leaf nitrogen and phosphorus, (Reich *et. al;* 1991, 1997). For example, if evergreen leaves with a lifespan of one year are favored in the wet tropics and in short growing seasons at high latitudes, but deciduous species are favored at mid latitudes (Kikuzawa *et. al;* 1996, Givnish *et. al;* 2002), this variation would decrease nitrogen and phosphorus at the highest and lowest temperatures, because species with longer-lived foliage have lower nitrogen and phosphorus (Reich *et. al;* 1991, 1997). However, for evergreen species, leaf lifespan is greater in colder environments (Reich *et. al;* 1996, 2003) given that leaf nitrogen and phosphorus increase with decreasing leaf lifespan, this trait should lead to lower leaf nitrogen and phosphorus in evergreen species in increasingly cold climates. Hence, variation in leaf life-span of evergreen species and the relative balance of evergreen and deciduous species could counteract one another in the tropics and at high latitudes.

The pattern of distribution of plant species was not uniform according to altitude due to variation in micro-climate. The highest number of species was found in site C. This may be due to the transition zone and this site acts as eco-zone. But in site A comparatively lower no. of species were reported and the forest was less dense in comparison to site B and site C, this indicate the forest has been intensively used long time for various forest products. And also might due to the mature forest with almost closed canopy and trees were large; so the number of species was low. In case of site B, *R. arboreum* along with *Q. semecarpifolia* and *Q. lanata* as dominating species in Shivapuri National Park. It may be due to the different topography and edaphic factors (Krebs 1972).

Species richness increased with soil moisture at lower elevation, but decreased with soil moisture at higher elevation. The abundance of the dominant plant species *Quercus semecarpifolia* at Shivapuri Nagarjuna National Park is due to the changes in temperature and water availability, along elevation gradients (Pausas and Austin, 2001). Another reason is due to the availability of road networks that increase resource extraction and encroachment into the forest leading to a reduction in biodiversity (Hitimana *et. al.,* 2004)

5.1 Specific leaf area:

In the present study, Specific leaf area was found decreasing with increasing altitude in both species i.e. *Q. semecarpifolia* and *Q. lanata* species(Fig. 5 and Fig. 6). With the increase in elevation, it is more difficult for plants to absorb soil nutrients with decreasing soil temperature, showing that plant growth slowed down and worked better in the stressed environment where retention of captured resources is a higher priority (Wilson *et. al.,* 1999).

Furthermore, the availability of nutrient element contents in plant leaves also result in the increase and decrease of specific leaf area which can be affected by the growth of the plants themselves (Baldwin *et. al.,* 2006). Increasing altitude with decreasing specific leaf area has been shown to be the most consistent morphological trend in leaves along altitudinal gradients (Korner *et. al.,* 1989).

Therefore, a decrease of specific leaf area allow individuals of *Q. semecarpifolia* and *Q. lanata* to maintain a relatively constant level of physiological activities along the altitudinal gradient. Also, low specific leaf area can limit the amount of water and carbon reaching the chloroplasts, and also further limit the photosynthesis rate (Li *et. al;* 2005).

5.2 Leaf Carbon Content:

The carbon storage was observed increasing at 1632m i.e. at (Site A) altitude but at middle 2020m (Site B) altitude and 2414m (Site C) altitude, (Fig.7 and Fig.8) it was observed at decreasing stage, the reason might be related with the absence of more productive leaf density between the first altitude than in middle and high altitude. Also an increasing altitude can be influence by different factors such as environmental factors i.e. altitude, temperature, population density, light, parasites, human disturbances, etc. which result in the declining of leaf carbon nutrients along the increasing altitudinal gradient (Sheikh, 2007). Greater carbon content may be associated with greater leaf rigidity required for longer persistence through resistance to climatic and mechanical stress and herbivore attack (Korner *et. al.,* 1983).

Also, with increasing altitude, environmental stresses such as solar radiation, temperatures, nutrients, and other abiotic pressures will increase, while photosynthetic ability and carbon assimilation will decrease, together resulting in an obvious decrease in leaf weight (Sheikh *et. al;* 2012). Large individual trees always account more carbon proportion. This study has shown variation on carbon storage the reason might be the area is affected by one of environmental variables i.e. altitude, followed by canopy cover, species diversity etc. (Mwakisunga *et. al;* 2012).

5.3 Leaf Phosphorous Content:

The study shows phosphorus was found high at 2020m(Site B) altitude, the reason might be due to the difference in the distribution of altitudinal pattern of plant and soil nutrient status among three 1632m, 2020m, 2414m (Site A, Site B, Site C) altitudes (Fig.11 and Fig.12). Phosphorus content was found less in senescence leaf of *Quercus semecarpifolia*, the reason might be due to the low supply of phosphorus in older leaves as they move their nutrients into other tissues from one plant tissues part to another (macgillivary, 1927). Also, the high mobility of phosphorus and the rapid rate with which it may move out of leaves and growing tissues when the external supply is depleted. The expansion and senescence dynamics of upper leaves were little affected by soil phosphorus level (Biddilph, 1941).

Also, several factors influence the availability of phosphorus along altitudinal gradients. Since, the functional substances stored in the plant are transported to new developed tissues, the leaf nitrogen and phosphorus contents were relatively high at the beginning of the growing season.

Then, nitrogen and phosphorus contents dropped significantly because of the dilution effect in the plant itself (Rong, 2014).

Furthermore, since the research sites at higher altitudes experience lower temperatures, *Quercus lanata* species experiences high phosphorus content than *Quercus semecarpifolia* species plants at higher altitudes it may be due to accumulation of more phosphorus in *Q. lanata* leaves in order to maintain their photosynthesis at low temperatures, since low temperatures reduce phosphate recycling (Savitch *et. al.*, 1997; Strand *et. al.*, 1997; Sage and Kubien, 2007). The combination of higher phosphorus availability and requirement would therefore lead to higher foliar phosphorus levels.

The leaves of evergreen species have a long life and low nutrient demand for nitrogen and phosphorus. Phosphorus is required to control the turgidity of stomatal guard cells that regulate gas exchange through the stomata, which in turn affects photosynthesis. Therefore, higher leaf phosphorus may provide more effective control over transpiration and allow for rapid growth when conditions are favorable (Schroeder *et. al.*, 2001).

5.4 Leaf Nitrogen Content:

The both species of *Quercus semecarpifolia* and *Quercus lanata* leaves shows decreasing trend along the different life stages (Fig. 9 and Fig.10) (immature, mature and senescence) it may be due to the reason that at evergreen woody plants leaf nitrogen show opposite responses to temperature(Shi, 2012). Also, *Quercus lanata* shows decreasing and at a sometimes found increasing at altitudinal gradient, leaf nitrogen increases as temperature decreases because high leaf nitrogen can offset reduced biochemical reaction rates caused by the diminished efficiency of nitrogen-rich enzymes at low temperatures . Also, low leaf nitrogen at leaves of both the species may be favored by cold climates because low temperature reduces the rates of decomposition and mineralization of organic matter, resulting in low soil-nitrogen availability (Reich, 2004).

The temperature-plant physiological hypothesis predicts that leaf nitrogen should raise with decreasing temperature, as high leaf nitrogen may compensate for the low efficiency of physiological processes at low temperatures (Reich and Oleksyn, 2004). Increasing leaf nitrogen can enhance photosynthetic capacity and osmotic pressure of a cell, facilitating the conservation of water inside the plant. Low specific leaf area can limit the amount of water and carbon-dioxide reaching the chloroplasts, and also further limit the photosynthesis rate (Li *et.*

al; 2005). So, a decrease of specific leaf area and an increase of leaf nitrogen allow individuals of *Q. semecarpifolia* and *Q. lanata* to maintain a relatively constant level of physiological activities along the altitudinal gradient.

Nitrogen is generally considered the most limiting element for terrestrial vegetation. The biogeography of leaf nitrogen (N) is a challenging issue and has drawn great attentions (He JS *et. al;* 2006). Hence, plant functional type and that leaf nitrogen variation was explained more by precipitation than by temperature at higher altitudes (Han *et. al;* 2012). Climate and vegetation-type change markedly with altitude over a short distance, therefore, mountains are ideal sites for examining the bio-geographical pattern of leaf nitrogen and the effects of climate, soil, and plant species on leaf chemistry.

5.5 Stomata Observation:

Stomatal size and density are known to change in response to a variety of environmental factors, including temperature, water availability, light, soil nutrients humidity and both the partial pressure and atmospheric concentration of carbon-dioxide (Carins Murphy *et. al.,* 2014). We found no significant relationships between leaf area or any of the stomatal variables with elevation but was significant between the considered plant species.

Variation in size and density of stomata may arise due to genetic factors and/or growth under different environmental conditions. Leaf morphological traits, including stomatal density and distribution, and epidermal features may affect gas exchange quite remarkably and their relationships with key environmental factors such as light, water status, and CO_2 levels (Woodward, 1987; Nilson and Assmann, 2007).

The differences in stomata numbers of the same leaf were small, and had many stomata in the leaf. Stomata are small but very numerous and the guard cells may cover more than fifty per cent. Stomata counts were made from different elevations on two species of oaks, *Q. semecarpifolia* and *Q. lanata* taken from all sides of exposed trees.

There was a marked variation in the density of stomata in the plant leaves as compared to both species. The density of stomata was found higher in the *Q. lanata* plant leaves than those of *Q. semecarpifolia*. The higher number of stomata was also observed in *Q. lanata* plant than in *Q. semecarpifolia* plant.

Leaf traits are affected by environmental conditions such temperature, precipitation, nutrient availability, carbon-dioxide availability and solar radiation (Roa *et. al.*, 2015). Controlling gas exchange depends on both stomata density (SD) and stomata length (SL) and is achieved through changes in aperture caused by external and internal factors not still entirely understood (Nishimura and Kim, 2010). The density and size of stomata are controlled through leaf development, allowing plant species to respond to local environments (Nadeau and Sack, 2002). Environmental conditions may affect leaf characteristics such as the stomata density even within individual plants. Commonly, leaves in full sun tend to have higher stomatal densities than leaves produced in shade conditions within the same individual (Li *et. al.*, 2006).

6. Conclusion:

Plant functional traits are the dimension of ecological strategy variation and these changes in plant functional traits along gradients is useful for understanding the assembly of communities and their response to global and local environmental drivers. Patterns of plant functional trait variation inform not only on community structure but also on population dynamics and the mechanisms affecting ecosystem functioning. Shifts in plant functional traits within or between sites and/or species across environmental gradients may also reflect deterministic processes of community organization.

Environmental gradients may influence a plant's physiological status and morphology, which in turn may affect plant- interactions. the relationship between environmental variation, physiological and morphological variability of plants in mountain ranges in dry environments have opposing altitudinal environmental gradients of temperature and aridity, which limit plant growth at high and low elevations. In an observational study, a series of functional traits as indicators of its physiological status (e.g. specific leaf area, nitrogen, carbon, phosphorus, stomata) were taken along the entire elevation range. Variation in functional traits among different leaf habits (deciduous and evergreen) may directly drive functional diversity in a community. Species with a low specific leaf area tend to have low nutrient concentrations (e.g., leaf nitrogen content, leaf phosphorus content), slow maximum photosynthesis and dark respiration rates.

Altitudinal variation, climatic conditions and disturbance most strongly influence trait expression in the study area. The study showed that altitude as well as different life stages (immature, mature and senescence) of *Q. semecarpifolia* and *Q. lanata* leaves is significantly influenced and shows effect on specific leaf area, leaf nitrogen content, leaf carbon content, leaf phosphorus content as the value was continually changing.

Leaf traits are important indicators of plant physiology and critical components of adaptation in an environment. Leaf traits and their adaptive behavior to the environment in context to the global climate change helps to understand the status and structure of forest community. Leaves are the important phenomenon in the life processes of plants within the leaf structure, shape, area, dry mass, number, size and position of stomata. The present study showed specific leaf area, leaf nitrogen content and leaf phosphorous content varies with the altitude and among the considered species *Q. semicarpifolia* and *Q. lanata* significantly which is most important component of a plant's photosynthetic apparatus and play a vital role in plant function and long-term adaptation to environmental change (Liu *et al;* 2016). Stomatal number and density is higher in *Q. lanata*. Various leaf parameters and leaf traits are important to maintain and modify community structure of forest.

Along altitudinal gradients, stomata densities within species often increase with altitude because of the lower partial pressure of carbon-dioxide at high altitude. Elevation and temperature have a strong impact on species composition and leaf traits in mountain systems. The vegetation also varies based on the altitude, so as the altitude changes, species composition changes, the changes in vegetation that occur along a water availability has a huge influence on leaf traits such stomata density, leaf area and stomata length as the evaporative cooling are largely removed along the altitudinal gradient.

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ANNEX-I

Dependent variable: Specific leaf area							
(I) Altitudes			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1632m	2020 m	173.35	669.67	0.796	-1143.83	1490.55
		2414 m	-1040.96	669.67	0.121	-2358.15	276.23
	2020 m	1632m	-173.35	669.67	0.796	-1490.55	1143.83
		2414 m	-1214.31	669.67	0.071	-2531.51	102.87
	2414 m	1632m	1040.96	669.67	0.121	-276.23	2358.15
		2020 m	1214.31	669.67	0.071	-102.87	2531.51
*. The mean difference is significant at the 0.05 level.							

Dependent Variable: Specific leaf area							
(I) Life Stage			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Senescence	immature	-1212.90	669.67	0.071	-2530.10	104.28
		mature	171.90	669.67	0.798	-1145.28	1489.10
	immature	Senescence	1212.90	669.67	0.071	-104.28	2530.10
		mature	1384.81*	669.67	0.039	67.62	2702.01
	mature	Senescence	-171.90	669.67	0.798	-1489.10	1145.28
		immature	-1384.81*	669.67	0.039	-2702.01	-67.62
*. The mean difference is significant at the 0.05 level.							

ANNEX-2

Dependent Variable: Carbon							
(I) Altitudes			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1632m	2020 m	-0.02	0.050	0.650	-0.12	0.07
		2414 m	-0.01	0.050	0.731	-0.11	0.08
	2020 m	1632m	0.02	0.050	0.650	-0.07	0.12
		2414 m	0.005	0.050	0.912	-0.09	0.10
	2414 m	1632m	0.01	0.050	0.731	-0.08	0.11
		2020 m	-0.005	0.050	0.912	-0.10	0.09
*. The mean difference is significant at the 0.05 level.							

Dependent Variable: Carbon							
(I) Life Stage			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Senescence	immature	-.155*	0.050	0.002	-0.255	-0.055
		mature	-0.053	0.050	0.292	-0.154	0.046
	immature	Senescence	.155*	0.050	0.002	0.055	0.255
		mature	.101*	0.050	0.047	0.001	0.202
	mature	Senescence	0.053	0.050	0.292	-0.046	0.154
		immature	-.101*	0.050	0.047	-0.202	-0.001
*. The mean difference is significant at the 0.05 level.							

ANNEX-3

Dependent Variable: Nitrogen							
(I) Altitudes			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1632m	2020 m	11.18^{*}	1.005	0.000	9.20	13.15
		2414 m	5.30^{*}	1.005	0.000	3.32	7.28
	2020 m	1632m	-11.18^{*}	1.005	0.000	-13.15	-9.20
		2414 m	-5.87^{*}	1.005	0.000	-7.85	-3.89
	2414 m	1632m	-5.30^{*}	1.005	0.000	-7.28	-3.32
		2020 m	5.87^{*}	1.005	0.000	3.89	7.85
*. The mean difference is significant at the 0.05 level.							

Dependent Variable: Nitrogen							
(I) Life Stage			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Senescence	immature	-6.952^{*}	1.005	0.000	-8.930	-4.974
		mature	-4.500^{*}	1.005	0.000	-6.478	-2.522
	immature	Senescence	6.952^{*}	1.005	0.000	4.974	8.930
		mature	2.452^{*}	1.005	0.015	0.473	4.430
	mature	Senescence	4.500^{*}	1.005	0.000	2.522	6.478
		immature	-2.452^{*}	1.005	0.015	-4.430	-0.473
*. The mean difference is significant at the 0.05 level.							

ANNEX-4

Dependent Variable: Phosphorus							
(I) Altitudes			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1632m	2020 m	-1.08*	0.294	0.000	-1.66	-0.50
		2414 m	0.03	0.294	0.911	-0.54	0.61
	2020 m	1632m	1.08*	0.294	0.000	0.50	1.66
		2414 m	1.11*	0.294	0.000	0.53	1.69
	2414 m	1632m	-0.03	0.294	0.911	-0.61	0.54
		2020 m	-1.11*	0.294	0.000	-1.69	-0.53
*. The mean difference is significant at the 0.05 level							

Dependent Variable: Phosphorus							
(I) Life Stage			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	Senescence	immature	-0.232	0.294	0.430	-0.812	0.346
		mature	-0.349	0.294	0.236	-0.928	0.230
	immature	Senescence	0.232	0.294	0.430	-0.346	0.812
		mature	-0.116	0.294	0.692	-0.696	0.462
	mature	Senescence	0.349	0.294	0.236	-0.230	0.928
		immature	0.116	0.294	0.692	-0.462	0.696
Based on observed means. The error term is Mean Square(Error) = 5.208.							