

**STUDY OF PLANT WATER RELATIONS AND  
PHENOLOGY IN *QUERCUS LEUCOTRICHOPHORA* A.  
CAMUS AND *CASTANOPSIS TRIBULOIDES* (SM.) A. DC.  
AT SHIVAPURI NAGARJUN NATIONAL PARK,  
KATHMANDU**



**A DISSERTATION SUBMITTED FOR THE PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE MASTER'S DEGREE IN BOTANY**

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**KATHMANDU, NEPAL**

**March, 2022**

## DECLARATION

I, Sarita Dhakal, hereby declare that the dissertation work entitled “**Study of plant water relations and phenology in *Quercus leucotrichophora* A. Camus and *Castanopsis tribuloides* (Sm.) A. Dc. at Shivapuri Nagarjun National Park, Kathmandu**” is a genuine work done by me and has not been published elsewhere for the award of any degree. All the information cited in this piece of work are specifically acknowledged and credited to the respective authors or institutions as references.



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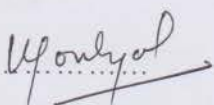
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## RECOMMENDATION

This is to certify that Ms. Sarita Dhakal has completed the dissertation work entitled "Study of plant water relations and phenology in *Quercus leucotrichophora* A. Camus and *Castanopsis tribuloides* (Sm.) A. Dc. at Shivapuri Nagarjun National Park, Kathmandu" under my supervision. The entire work is based on the results of her own field work and laboratory work and has not been submitted in any other academic degree to the best of my knowledge. I, therefore, recommended this dissertation to be accepted for partial fulfillment of Master's Degree in Botany from Amrit Campus, Tribhuvan University.

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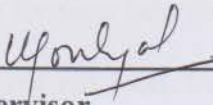
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## LETTER OF APPROVAL

This dissertation work entitled “Study of plant water relations and phenology in *Quercus leucotrichophora* A. Camus and *Castanopsis tribuloides* (Sm.) A. DC. at Shivapuri Nagarjun National Park, Kathmandu” submitted by Sarita Dhakal has been accepted for the examination and submitted to Amrit Campus, Tribhuvan University for partial fulfillment of the requirements for Master’s Degree in Botany (Ecology).


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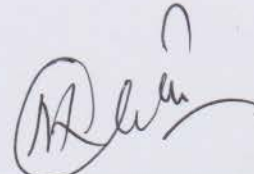
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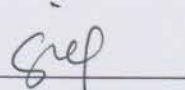
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*Sarita*  
Sarita Dhakal



## ACRONYMS AND ABBREVIATIONS

$A_l$	Leaf surface area
ABA	Abscissic acid
$A_{we}$	Effective xylem area
$A_{wt}$	Mean xylem area
BP	Balance pressure
<i>et al.</i>	And others
$\Psi_w$	Water potential
$\Psi_{Lpd}$	Predawn leaf water potential
$\Psi_{Lmd}$	Midday leaf water potential
$\Psi_{pd}$	Predawn water potential
$\Psi_p$	Pressure potential
$\Psi_s$	Osmotic potential
$\Psi_{sf}$	Osmotic potential at full turgor
$\Psi_{sz}$	Osmotic potential at zero turgor
$\Psi_m$	Matric potential
$\Psi_g$	Gravitational potential
$g_w$	Stomatal conductance
$H_v$	Huber value
$\epsilon$	Index of elasticity
IDT	Index of desiccation tolerance
$K_h$	Hydraulic conductivity
$K_l$	Leaf specific conductivity
$K_s$	Specific conductivity
MPa	Mega Pascal
PG	Pressure gradient

P-V curve	Pressure volume curve
PWP	Permanent wilting point
$r$	Karl Pearson coefficient of correlation
RWC	Relative water content
$RWC_z$	Relative water content at zero turgor
SLM	Specific leaf mass
SMC	Soil moisture content
SNNP	Shivapuri Nagarjun National Park
Starch <sub>0</sub>	Starch content in zero year twigs
Starch <sub>1</sub>	Starch content in one year twigs
VDC	Village Development Committee

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## ABSTRACT

Study on plant water relations and phenology of *Castanopsis tribuloides* and *Quercus leucotrichophora* was performed in Shivapuri Nagarjun National Park, Kathmandu, Nepal. Monthly variation in water relation parameters and phenological processes such as leaf emergence, senescence, flowering, fruiting, etc. of *C. tribuloides* and *Q. leucotrichophora* were studied. The measurements were carried out from February 2019 to January 2020 at monthly interval. Water relation parameters like soil moisture content at 15 cm and 30 cm depth, Pressure Volume (P-V) curve analysis, Huber value ( $H_v$ ), specific conductivity ( $K_s$ ), leaf specific conductivity ( $K_l$ ), hydraulic conductivity ( $K_h$ ), specific leaf mass (SLM), twig starch content and phenological changes were recorded. Soil moisture content showed significant variation among sampled months and was lowest in the month of February. Relative water content at zero turgor ( $RWC_z$ ) showed lowest value during March in both species. Both osmotic potential at zero and full turgor ( $\psi_{sz}$  and  $\psi_{sf}$  respectively) showed lowest value during November in *C. tribuloides* and during January in *Q. leucotrichophora* when post monsoon rain was negligible. High elasticity in *C. tribuloides* was found in April and showed a synchrony with the ongoing phenological activities. Phenological activities such as bud break, leaf emergence, twig emergence and shoot elongation in both species started with the onset of dry months (February-May) while flowering in *Q. leucotrichophora* started in dry months but in *C. tribuloides* flowering took place in the month of September. Low values in Relative Water Content at zero turgor ( $RWC_z$ ), osmotic potential at zero turgor ( $\psi_{sz}$ ), xylem conductance and index of elasticity ( $\epsilon$ ) were found during dry months in both species in spite of high IDT. Xylem conductance had a strong effect on phenological activities. Some patterns of xylem conductance were related to leaf emergence as  $K_h$ ,  $K_l$  and  $H_v$  increased in February.  $Starch_0$  and  $starch_1$  in both species showed high seasonal variation and had a strong effect of phenological activities. SLM decreased at the time of senescence and increased when leaves were fully expanded and mature.

**Keywords:** Huber value, leaf specific conductivity, osmotic potential, phenology, relative water content, specific leaf mass, xylem conductance.

# CHAPTER 1: INTRODUCTION

## 1.1. Background

Water, a substance that may justifiably be called the fluid of life, is crucial for all plants as an integral part of their living systems both ecologically in shaping environmental conditions patterns and biochemically as an important aspect in physiological processes (Manske, 2001). Water is commonly considered to be the most important environmental factor affecting growth and distribution of trees (Hinckley *et al.*, 1991).

Plant water relations explain how plant control the hydration of their cells, including the availability of water from soil, its transport within the plant and its loss by evaporation from the leaves. Plant function requires effective mechanisms such as photosynthesis, transpiration, support system and nutrient distribution. A physiological necessity for all protoplasm, water, from the ecological viewpoint, is chiefly a limiting factor in land environments. It is also necessary as a transport medium in the xylem and phloem and helps in the maintenance of the shape and orientation of cells and stomatal function (Hinckely *et al.*, 1991). Water availability is a major factor controlling vegetation development and species distribution (Kramer and Boyer, 1995). Growth of plants particularly depends on at least a minimum degree of cell turgor.

Independent of the species, plant requires a water volume from the soil that overcomes its metabolic necessities. Decrease in water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, stomatal closure, reduction in photosynthesis and interference with many other basic metabolic processes (Kramer and Boyer, 1995). Tree water status varies with availability of subsoil water and a variety of physiological and structural factors such as life span of leaves, time of leaf shedding, wood density, stem water storage capacity and the depth and density of root systems (Borchert, 1993). Growth of higher plants is more sensitive to their water supply and responds more quickly to soil water deficit than to any other factors of the environment.

Soil water content is the amount of water stored in the soil at a given time. The most commonly defined soil water content values are saturation, field capacity, wilting

point, etc. Plants absorb water through root hairs from the soil which can sense moisture gradient and grow toward moist patches (Lambers *et al.*, 1998). Leaves receive the signal of soil drying through phytohormone Abscisic acid that reduces the stomatal conductance and leaf growth even when the leaf water status is normal (Passioura, 1988). As the soil dries, plants sense to water limitation increases and water uptake by roots diminishes. As metabolism in plant cells decrease, the cells synthesize osmotically compatible solutes in the cytosol, mainly sugars and amino acids such as sorbitol, mannitol, proline and some ions. The accumulation of these solutes reduces the solute potential as well as water potential of cell which being more negative, enables the plant to maintain a positive turgor pressure (Lambers *et al.*, 1998).

Hydraulic lift is the movement of water from deep moist soil to drier surface soils through root system. Primarily hydraulic lift occurs at night when stomata are closed, so that plant is at equilibrium with root water potential (Lambers *et al.*, 1998). The nocturnal hydraulic lift in deep rooting plants helps other plants with more superficial root systems to survive period of drought (Larcher, 1995).

The structure of the water conductive system (hydraulic architecture) can potentially limit leaf water potential, stomatal behavior and gas exchange (Tyree and Ewers, 1996). Stomatal behavior is probably the most sensitive and earliest indicator of the physiological status of the whole plant (Smith and Hollinger, 1991) and depends both on the water availability in the soil and on the vapour pressure of the air (Lambers *et al.*, 1998). Plant controls their water status by regulating the size of the stomatal openings, which modify the rate of water loss thus reducing water loss can reduce the effect of deficient soil moisture on water potential (Zobel and Singh, 1995).

The hydraulic conductivity is important in determining the water potential ( $\psi_w$ ) of a plant in the field. The amount of water moved through the vascular system in unit time depends on the specific properties of the xylem, such as the conducting area (cross sectional area of the vessels) and flow resistances; on the physiological state of the plant, e.g. degree of the stomatal opening; and on environmental conditions (Larcher, 1995).

In general, xylem is an evolutionary structure compromising between efficient conduction and protection against blockage by gas bubbles (embolism), being most efficient for conduction of water but more at risk to embolism formed by cavitations (Kozlowski *et al.*, 1991). A drop in the water potential inside xylem vessels below a threshold value causes cavitation of water in xylem conduits. Xylem embolism by cavitation causes a diminish in hydraulic conductance of plant stems (Tyree and Sperry, 1989), which will in the long run cause reduction in photosynthetic rates and biomass generation due to closure of stomata.

An analysis of the changes in water potential, which actually is the osmotic potential of the vascular sap and the turgor or pressure potential (Kozlowski *et al.*, 1991), can be used to determine several properties of the plant that may affect its water relations. Pressure-volume analysis is the tool that is used in analyzing of various components of tissue water relation parameters and their relationship with tissue relative water content (Tyree and Jarvis, 1982).

Turgor maintenance which is critically important for plant growth and survival under severe drought condition as in Himalayan trees, is achieved mainly by the integrated mechanism of osmotic potential, changes in tissue elasticity (bulk modulus of elasticity) and tissue relative water content (RWC). Deciduous species, which could reduce relative water content (RWC) to lower value before turgor loss, have more elastic tissue and higher osmotic potential at zero turgor ( $\psi_{sz}$ ) and osmotic potential at full turgor ( $\psi_{sf}$ ) than evergreens. This supports the suggestion that elastic cell walls are important for drought resistance in trees (Lambers *et al.*, 1998) but appears to contrast Davis (2005) who showed that a less elastic cell wall was also important in drought resistant trees. It appears that elastic walls allow tissue to maintain turgor longer as water is lost, while the stiff (less elastic) walls cause  $\psi_w$  to drop quickly as water losses, increasing the gradient of  $\psi_w$  from soil to leaf and increasing water uptake (Poudyal *et al.*, 2012a).

Trees subjected to low water potential during leaf growth reduce osmotic potentials and increase the leaf elasticity during that period. When  $\psi_{sf}$  is low, plants can maintain turgidity at low relative water content at zero turgor (RWC<sub>z</sub>). Osmotic adjustment of this kind has been reported in some oaks (e.g., *Quercus petraea*) where soil drying induced the accumulation of fructose and glucose (Epron and Dreyer,

1996). Osmotic adjustment could enable the plant to maintain turgor at lower  $\psi_w$  and continue to absorb water from relatively dry soil (Lambers *et al.*, 1998). Also the stomatal aperture is controlled by osmotic adjustment in the surrounding cells.

Moreover, changes in an index of tissue elasticity ( $\epsilon$ ) are more significant for adaptation to drought than osmotic adjustment (Niinemets, 2001). However, the role of elasticity in turgor maintenance is not well-defined (Clifford *et al.*, 1998, Niinemets, 2001). Either low or high elasticity might benefit plant performance, which depends on different situations. The high elasticity in soft, developing leaves would help to maintain their turgor before the monsoon. Transpirational demand during leafing may be decreased by leaf loss that begins well before new leaf expansion, a short period of leaflessness in some years by Himalayan evergreen species (Negi & Singh 1992, Poudyal *et al.*, 2004), or completion of leaf expansion during the monsoon in *Pinus* (Zobel and Singh, 1995) and on *Quercus* sp. (Poudyal *et al.*, 2004).

Leaf structural character influences leaf energy and water balance. Specific leaf mass (dry leaf mass per area) is a key quality in plant development (Lambers and Poorter, 1992). A higher SLM has a higher energy cost (Griffin, 1994) and requires a longer time for leaf expansion. Longer photosynthetic periods probably compensate for the high energy cost of evergreen leaves, with their high SLM (Kikuzawa, 1995). Evergreen leaves lose mass gradually, without senescing, during late winter and spring. But in contrast, deciduous leaves begin to lose mass before the end of rainy season and senesce rapidly during autumn (Ralhan and Singh, 1987). Spiny leaves might be associated with their drought tolerant adaptations. Spiny leaves in *Q. semecarpifolia* young plants when drought was severe have been reported by (Poudyal *et al.*, 2012b).

Starch, a photosynthetic storage of plants is accumulated when sucrose production exceeds the demand than it is required by the plants. Since any kind of stress, external or internal, decreases the photosynthetic rate of plant tissues reduce the starch in plant. Relative amount of starch in one and two years old twig of dominant trees have been successfully used to detect stress on forest (Zobel, 1996a; Poudyal *et al.*, 2012b). Mostly a higher starch staining was found in zero year twigs than in the previous year's twigs in *Castanopsis indica* which indicated the expeditious shift of such



accumulates to the new shoots (Poudyal, 2014). Decrease in starch staining after leaf emergence indicated hydrolysis of photosynthates into soluble carbohydrates (Poudyal, 2014). The seasonal variations may show a significant correlation with water relation parameters and phenology of trees.

Phenology is the timing of biological event and their relationship to seasonal climatic changes. The biological events such as bud burst, flowering, fruitification, leaf senescence and leaf fall, are genetically pre-determined but also strongly modulated by environmental conditions. Phenological events of the plants are good indicators of climatic differences and can be used to monitor the biotic and local climate characteristics in plants species (Orshan, 1989). In seasonal climate, trees often experience water deficiency and hence seasonality of moisture deficit is reflected in their physiological mechanisms and phenological processes. The onset and duration of particular phases of development vary from year to year, depending on the weather (Larcher, 1995). Thus, phenological observations provide background information on the functional rhythms of plants and plant communities (Singh and Singh, 1992; Poudyal *et al.*, 2012b). Many researchers have observed phenological variations as a consequence of tree water status: leaf expansion (Meinzer *et al.*, 1983), bud set and shoot elongation (Reich and Borchert, 1984), premature leaf senescence, bud burst and flowering of trees (Borchert 1994a, b and c).

Phenology, the distribution of plant activities in time, is associated with soil and tree water status (Borchert, 1994a). The phenological behavior of plants is generally affected by the interaction between water availability, stem water storage and sensitivity to water stress (Holbrook *et al.*, 1995). In Himalayan trees, the phenological processes such as leaf emergence, expansion, flowering and fruiting takes place before the onset of monsoon when trees still bear old leaves primarily at the end of the dry season (Poudyal *et al.*, 2004) indicating that the phenological activities are not inhibited by moderate water stress as found in other part of world (Meinzer *et al.*, 1983) but indicates that the evergreen tree cannot rehydrate during a leafless dry season, as Borchert (1994a, b, c) describes for American dry tropical trees. Evergreen tree species of central Himalaya have leaf drop mainly on dry summer seasons with simultaneous leafing (Ralhan *et al.*, 1985; Poudyal, 2014). The deciduous species which generally occur in the forests of lower elevations also leaf

out during the dry summer season (Shrestha *et al.*, 2007) when day length is increasing and temperatures are rising.

Leaf phenology in both seasonal and non-seasonal environments is a major element in plant strategies for carbon gain (Kikuzawa, 1995). Phenology is a timing of biological events in plants such as leaf flushing, leaf fall, bud break, flowering, fruiting, etc., and their relationship to seasonal climatic changes (Leith, 1974), as the relationship between climatic factors and seasonal biological events showed the phenological changes in plants (Barbour *et al.*, 1999). Seasonal variation in water status not only determines phenology but also the distribution of trees and forest composition (Borchert, 1994a and Zobel *et al.*, 2001).

The natural vegetation of the central Himalaya grow in a particular environmental conditions compared to other parts of the world. There is concentrated rainfall during the monsoon from mid-June to mid-September. Such concentrated warm season and the subsequent 8-9 months of dry periods, limited variation in day length and year around moderate temperature has led to observe the variations in the phenological events (Poudyal *et al.*, 2012b). Most of the Himalayan trees are phenologically active during the dry season i.e. March to May (Ralhan *et al.*, 1985, Poudyal *et al.*, 2012b). In Himalaya trees, both evergreen and deciduous, timing of development and senescence of leaf have been linked to variations in the tree water relations (Escudero and Del Acro, 1987) and such observation strongly plays a dominant role in structuring temporal pattern of the expansion and senescence of leaves.

## **1.2. Justification**

The natural vegetation of the central Himalaya grows in a particular environmental conditions compared to other parts of the world. There is concentrated rainfall during the monsoon from mid-June to mid-September. Such concentrated wet season and the subsequent 8-9 months of dry periods are likely to have profound effects on plant adaptation and ecosystem processes (Singh and Singh, 1992, Zobel and Singh, 1997). So water may become the limiting factor in many sites in Nepal.

For the phenological processes such as leaf emergence, expansion, flowering & fruiting that occur before the onset of monsoon when trees still bear old leaves where rehydration of leafless twigs becomes a prerequisite for initiation of new leaves

(Borchert, 1994a). In Himalayan trees, the phenological processes take place primarily at the end of the dry season (Poudyal *et al.*, 2012a,b) indicating that the phenological activities are not inhibited by moderate water stress as found in other part of world (Meinzer *et al.*, 1983). In recent years, research has suggested that forests in certain areas might become more vulnerable to drought in the close future not only due to water depletion but because of the amplified effect of changes in climate variability and extremes resulting from global warming.

The role of environment, particularly drought, in controlling the distribution and performance in Himalayan tree species is poorly understood (Zobel and Singh, 1997, Poudyal, 2004). Thus, two evergreen tree species namely *Q. leucotrichophora* and *C. tribuloides* were selected to understand their response and adaptive strategy to drought and relationship between the different attributes of water relations parameters and their different phenological events.

### **1.3. Research objectives**

The main objective of this research study is to find out the relative importance of plant water potential for drought resistance and completion of phenological activities during dry periods.

The specific objectives are as follows:

1. To measure starch reserves in twigs and its relation with water relations & leaf development.
2. To determine soil moisture content.
3. To analyze pressure-volume curve to find components of water potential i.e. relative water content, osmotic potential, index of tissue elasticity and desiccation tolerance.
4. To estimate the specific leaf mass.
5. To measure twig conductance i.e. Huber value, hydraulic conductivity, specific conductivity & leaf specific conductivity.
6. To record the phenomorphological events and relationship between phenology and different attributes of water relations of *Castanopsis tribuloides* and *Quercus leucotrichophora*.

#### **1.4. Limitations**

- Length of time and numbers of measurements are the limiting factor for this study because as a part of Master degree in Botany, time period is limited.
- No public transport facility. Private vehicles were very costly.
- Unprecedented rainfall during measurements.
- Disrupt in electric supply during lab work.

## CHAPTER 2: LITERATURE REVIEW

### 2.1. Historical Background

As far as known, Stephan Hales in 1727 had made the first quantitative experiment on plant water relations in “Vegetable Staticks”. He concluded that the rate of transpiration varies with the species, temperature, time of the day and irradiational gradient (Kramer and Boyer, 1995).

The finding of De Saussure (1804) was that the absorption of minerals by plants was not proportional to the absorption of water and roots differ in permeability to various solutes. Development of osmotic theory named ‘osmotic hypothesis’ in the middle of 19th century by Dutrochet (1837) was a significant development in plant water relations. This hypothesis was used to explain a variety of phenomena including the escape of spores from sporangia and the water uptake by plants exhibiting root pressures. Pfeiffer (1877) for the first time measured accurately osmotic pressure, using the membranes of Traube (1867).

Sachs (1882a, b) made great breakthrough in the development of concepts in modern plant physiology as well as plant water relations. His findings were related with the effects of soil mixture, soil aeration, and soil temperature on water absorption and root growth.

In the 20th century, Brown and Escombe (1900) described diffusion of water through stomata to be a purely physical process. In 1914, Dixon published the “Transpiration and Ascent of Sap in Plants”, which was a landmark in the study of water relations. Experiment by Stalfelt (1932) and Bange (1953) indicated that stomatal closure has a large effect on transpiration in moving air where boundary layer resistance is low.

The use of thermocouple psychrometers (Spanner, 1951; Monteith and Woen, 1958; Richards and Ogata, 1958) and pressure equilibration by (Scholander *et al.*, 1964) were the most important development in the water relation research that made the work easier and reliable. This method is widely used in the present context of water relation research by recent workers.

## 2.2. Recent works

The availability of soil water to the roots and the status of energy determine the growth and development of plants. In an experiment with *Zea mays* L. and *Helianthus annuus* L. in drying soil Zhang and Davies (1990) observed that reduction in the rate of leaf expansion in response to moisture deficit in soil would be an effective mean of limiting water loss during a prolonged drought. They also reported increased translocation of abscissic acid (ABA) from roots exposed to drying soil. Thus they considered ABA as playing major role in root-shoot signaling.

Abrams (1990) reviewed eco physiological studies on the tissue water relationship of different species of *Quercus*, growing successfully in drought prone sites of North America and concluded that various morphological and physiological traits such as root depths, effective soil to leaf conductance and lowering of osmotic potentials (osmotic adjustment) during the dry periods facilitate their tolerance to drought.

When the availability of water in the soil gradually depleted, leaf growth abolishes before photosynthesis and respiration are affected, however roots continued to grow rapidly in the same plant at the same tissue  $\psi_w$  which probably served to reduce the development of new transpiration surface and increase the access to soil water (Kramer and Boyer, 1995). As the soil dries, changes in root metabolisms (such as increase in ABA production and disturbance of nitrogen metabolism) send biochemical signals to the shoots which produce physiological changes such as reduction in growth, stomatal conductance and rate of photosynthesis regardless of the water status of the leaves.

Pre-dawn  $\psi_w$  is an important reflection of the amount of soil moisture available to plants. Pre-dawn tree  $\psi_w$  of *Pinus contrata* and soil  $\psi_w$  having contrasting soil water properties were measured in 1984 by Fahey and Young. They found large difference between predawn tree  $\psi_w$  and soil  $\psi_w$  in coarser soil with low diffusivity of water. In studying water relation of ash (*Fraxinus excelsior*), Carlier *et al.*, (1992) found the predawn water potential to be close to the soil water potential at 30cm depth. They also found that the maximum stomatal conductance decreases as soil dries up. Similarly, a greenhouse experiment conducted by Wang *et al.*, (1998) on four birch (*Betula papyrifera*) populations determined soil moisture as the major factor affecting



photosynthesis. Photosynthesis of trees in the low water and high nitrogen was about 45 percent of that in the high water and high nitrogen treatment.

In *Thuja occidentalis*, Edwards and Dixon (1995) observed significant lowering of water potential when subjected to drought. However, Thomas and Hartmann (1996) studied water relations in mature oak stands of North Germany and found that drying of soil had little impact in water relations of oaks. Fort *et al.*, (1998) concluded that short-term soil water depletion affecting only 50% of the root system did not show a measurable stress response in birch (*Betula pendula*) seedlings despite a growth cessation of roots in some part. In their study of influence of soil drying on leaf  $\psi$ , photosynthesis, stomatal conductance and growth in black pine varieties, Francois *et al.*, (1998) observed that these varieties exhibited drought avoidance strategy characterized by an efficient stomatal control of transpirational water loss.

Adhikari (2000) conducted a research on water relations of *Castanopsis tribuloides* (Sm.) A.DC. and found soil water potential to be lowered from mid-winter (January) to mid-summer (April). The lowest water potential was found in middle summer and highest in late monsoon. Whereas lowest diurnal change in leaf water potential was found in late summer (May) and highest in early summer and early monsoon.

Kolb and Stone (2000) comparatively studied gas exchange and water relations in Gambel oak (*Quercus gambelli* Nutt.) and ponderosa pine (*Pinus ponderosa* var. *scopulorum* Dougl. ex. Laws.) and observed higher predawn leaf  $\psi$ , stomatal conductance in oak than pine which they attributed to greater avoidance of water stress in oaks due to extensive rooting system.

Tewari (2000) observed the relation between predawn  $\psi$  and leaf canopy for two years in *Quercus leucotrichophora* and he found insignificantly higher  $\psi$  in the latter year when leafing was less as compared to former year, which he explained as thinning of canopy which brings about the changes in the internal water status of the whole plant. This work was in agreement with that of Williams *et al.*, (1997) in Australian savanna.

In a study carried out by Bhattarai (2001) in *Schima wallichii*, soil water potential, tree water potential, leaf water potential and stomatal conductance showed a good positive correlation among themselves, and to the seasonal variations. These

parameters showed their minimum values during March, which was the driest month during the study period.

The water conducting system and xylem hydraulic conductance is affected by plant water potential. Stout and Sala (2003) made an experiment and found lower predawn  $\psi$  in ponderosa pine (*Pinus ponderosa*) than Douglas-fir which was more vulnerable to cavitation than the latter.

Poudyal (2013) studied soil water potential ( $\psi_w$ ), predawn and midday leaf water potential ( $\psi_{Lpd}$  and  $\psi_{Lmd}$ ) and their relationship with stomatal conductance ( $g_w$ ) and phenological behavior in *Q. semecarpifolia* and found high  $\psi_{Lpd}$  and  $g_w$  despite low soil  $\psi_w$ . Patterns of  $\psi_{Lpd}$  and  $\psi_{Lmd}$  correlated significantly with soil  $\psi_w$  and phenology as  $\psi_{Lpd}$  often increased during leafing but not with  $g_w$ .

Poudyal (2014) found that the seasonal patterns of precipitation showed profound effect on average  $\psi_{pd}$ . The reason for lower  $\psi_{pd}$  than soil  $\psi$  in winter months, when ground herbaceous vegetation almost dried out might be due to low temperature that decreased water movement from the soil into the roots thus reducing plant  $\psi$ . Besides the occasional rise in  $\psi_{pd}$  during the phenologically active periods, plants depended on their stem water storage for the rehydration during phenological activities. A similar increase of  $\psi_{pd}$  in *C. indica* was reported by Poudyal *et al.*, (2004) when trees were trimmed. Lopped trees can have higher  $\psi_{pd}$  than undisturbed trees (Singh and Thompson, 1995).

According to Chand *et al.*, (2017), predawn water potential  $\psi_{pd}$  and midday water potential  $\psi_{md}$  showed a profound effect of precipitation on it and low value was observed during the dry summer month. Species at higher elevation faced more water stress than at lower elevation as shown by their low leaf  $\psi$ .

Collinson *et al.*, (1997) studied the effect of soil moisture deficit on the water relation of bambara ground nut (*Vigna subterranea*) and found that the plant was able to maintain turgor through a combination of osmotic adjustment, reduction in leaf area index and effective stomatal regulation of water loss.

Relationship between water deficit and transpirational demand is unlikely to be stable during water stress. Monterio *et al.*, (2018) concluded that the water potential in the

peach tree branch has a positive relationship with the evaporative demand until the end of the peach harvest and a negative relationship with the soil water shortage. Similarly, Carlson *et al.*, (1994) studied plant- soil water relation on forestry and silvipastoral system in Oregon, USA and found that grazing of understory vegetation might reduce water stress of trees during dry periods by reducing transpirational water use by the forage plants. According to Dawson (1996) small trees had lower overall rates of water loss at leaf and canopy leaves than large trees.

Stomatal closure is a drought avoidance mechanism and this mechanism represents tradeoff between continued transpiration and photosynthetic activity (Levitt, 1972). Thus stomata have evolved as regulatory valves to minimize water loss for a given amount of carbon gained (Bradford and Hsiao, 1982).

Maier and Koch (1995) studied stomatal control and abscission of needles in spruce of Northern Alps and found the association of low leaf turgor values with shedding of green needles. The loss of stomatal control changed the drought avoidance abilities of the foliage and as a consequence, low turgor level occurred and premature needle abscission was induced.

Schiller and Cohen (1999) found that transpiration rate in pine dropped after last rain, the decrease being larger in smaller tree needles. Water potential at sunrise reduced from -0.8 MPa in the rainy season to more than -3.0 MPa during the dry season. Stomatal responses to water availability in soil, leaf and atmosphere are highly interactive and specific. Bond and Kavanagh (1999) studied four woody species and hypothesized that species differences in stomatal response to humidity and soil water availability can be explained by two parameters: leaf specific hydraulic conductance and threshold water potential. They constructed a simple hydraulic model based on these two parameters which predicted stomatal behavior that were similar to the published reports. Likewise, changes in water relations, gas exchange, and carbohydrates of white spruce seedling were studied by Wang and Zwiazek (1999). They observed that both net photosynthesis and stomatal conductance showed a significant linear relationship with minimum air and soil temperature, and there was a significant linear relationship between net photosynthesis and stomatal conductance.

Lowenstein and Pallardy (1998) studied three deciduous trees (*Juglans nigra*, *Acer saccharum* and *Quercus alba*) in Missouri, USA and showed the pattern of water relations, xylem sap abscissic acid concentration and stomatal aperture. They observed a strong correlation among reduction in predawn leaf water potential, xylem sap abscissic acid concentration and stomatal closure in all species.

Naor (2001) studied interrelationships between water potential and stomatal conductance in *Pyrus communis* L. A high correlation was observed between stomatal conductance and leaf water potential ( $r^2= 0.54$ ), but much better correlation was found with stem water potential ( $r^2= 0.80$ ). Decreasing RWC of leaves progressively decreased stomatal conductance, slowing CO<sub>2</sub> assimilation which eventually stopped, after which CO<sub>2</sub> is evolved (Lawlor, 2002).

The osmotic potential of the plant determines relationship between water potential and relative water content, by the proportion of water in the plant that is outside the living cells and by the elasticity of the cell walls. As internal water balance of cells and tissues is the most important aspect of plant water relation, which is expressed in terms of turgidity. All other parameters of water relation are important because they affect internal water balance of trees and thereby modify the physiological process and condition, which affects growth (Kramer and Kozlowski, 1960).

Dreyer *et al.*, (1990) conducted Pressure volume analyses on leafy shoots of 4 European oak species (*Quercus robur*, *Q. petraea*, *Q. pubescens* and *Q. ilex*) in order to determine the relationship between leaf water potential, average osmotic potential and volume averaged turgor. They found that tolerance to drought appeared to be related more to the ability to osmotically adjust in response to changes in environment rather than to the absolute values of full turgor.

Reed (2001) evaluates the osmotic potentials at various canopy positions and found that the older and taller trees are osmotically regulating themselves in order to maintain turgor. The cells appear to be adjusting their water status by synthesizing osmotically active solutes, which reduces  $\Psi_s$  and therefore helps to maintain turgor.

Martinez *et al.*, (2007) reported that osmotic adjustment which increases the osmotic force thus promoting water absorption is known as an effective mechanism of drought resistance and it provides a mean of maintaining cell wall water status. Similarly,

Zlatev and Lidon (2012) observed that the changes in elasticity of cell wall (elastic adjustment) in response to drought can modify the relationship between turgor pressure and cell volume that may contribute to drought tolerance. Likewise, Poudyal (2013) reported that elastic adjustment is more important for drought resistance than osmotic adjustment as higher elasticity indicates greater fundamental ability to shrink when dehydrated although both elastic and osmotic adjustment are important strategy to maintain turgor during drought stress. It was found that *S. wallichii* maintained turgor by both elastic and osmotic adjustment whereas *Q. semecarpifolia* only displayed osmotic adjustment to survive in the drought prone Himalayas.

Sailendra and Meinzer (1991) observed solute accumulation and increased elasticity as leaf water deficits developed more slowly during soil drying, in their work in droughted sugarcane. This osmotic and elastic adjustment maintained symplast volume essentially constant both diurnally and during soil drying, whereas turgor was only partially maintained. The extent of osmotic adjustment associated with drought was not reflected in the leaf  $\psi_{sf}$  because the concurrent increase in tissue elasticity resulted in a larger symplast volume at full turgor.

Premachandra *et al.*, (1992) observed osmotic adjustment in four *Sorghum bicolor* L. Moench cultivars under water deficit conditions. Lower  $\psi_s$  enabled plants to maintain turgor and decreased the sensitivity of turgor dependent process. The cultivars, which maintained higher turgor and higher osmotic adjustment under water deficit conditions, were identified as drought tolerant.

Fan *et al.*, (1994) made a comparative study of the role of osmotic and elastic adjustments in turgor maintenance of different woody species: jack pine (*Pinus banksiana*), black spruce (*Picea mariana* Mill.) and flooded gum (*Eucalyptus grandis* W. Hill) and found jack pine to lose turgor at the lowest relative water content (75-60%) and water potential among the three species and suggested a high inherent drought tolerance than the other two species.

Zobel *et al.*, (1995) studied agriculturally related disturbance in stand of *Quercus leucotrichophora* and showed lower specific area, higher predawn water potential, and larger level of disturbance as compared to lowest level.

In addition to water relation parameters and strategies that plant uses (eg. osmotic and elastic adjustment) to neutralize drought stress, plants have evolved many physiological, morphological, anatomical and phenological characteristics for responding to and resisting drought stress (Auge *et al.*, 1998).

Niinemets (2001) concluded that osmotic adjustment of leaf water relations is inherently limited, and that elastic adjustment resulting from changes in leaf structure may be a more important and general way for plants to adapt to water-limited environments. Zimmermann *et al.*, (2002) reviewed that pressure gradients, pressure potentials, ion concentration, volume of flow as well as turgor pressure gradient were the major driving forces for water lifting in the xylem conduit.

Water movement in plant takes place along the water potential gradient by diffusion from cell to cell (short distance transport) and by conduction (long distance transport) through the xylem and is transported in the xylem under tension (Dixon and Joly, 1894). Water transport through the xylem is essential to replace water loss during transpiration, thus avoiding desiccation and allowing photosynthesis. According to the study of Waring and Running (1978), the hydraulic conductivity has higher value for trunk than for branches and particularly low in second order branches and at branch insertions (Ewers and Zimmermann, 1984). It is controlled by wood anatomical features; water absorbed by root is transported to leaf through xylem. Hydraulic structure and its embolism have significant influence on drought tolerance. Wider conduits are more vulnerable to cavitation than narrower conduits. Although this relationship seems to be uncertain when different species and taxa are compared, it appears to be valid within one species (Tyree and Ewers, 1991).

According to Han and Kim (1996), the relative hydraulic conductance increases with decreasing diameter of the stem and branches. Leaf specific conductivity and specific conductivity decreased with decreasing diameter but Huber value slowly increased with decreasing diameter. Likewise, Tyree and Cochard (1996) examined summer and winter embolism in oak and its impact in water relation. They observed that the impact of embolism was reduction in hydraulic conductivity in the vascular system. Oaks seemed to operate close to the point of xylem dysfunction but they protected themselves against embolism by stomatal regulation which kept water potential above that causing runaway embolism.

Zwieniecki and Holbrook (1998) reported that xylem conductance decreases during the day and increases in the night so the sampling time may also be a reason for low or high hydraulic conductance beside the size of the stem diameter.

Hubbard *et al.*, (1999) observed the effect of xylem conductance on photosynthetic rate and productivity and proposed that decrease in net photosynthesis rate during tree maturation was due to decreased xylem hydraulic conductance.

Strong positive correlation between leaf specific conductivity and maximum photosynthetic capacity was observed by Brodribb and Field (2000) suggesting that maximum photosynthetic rate was constrained by their vascular supply. The leaf specific conductivity of conifer wood overlap broadly with that of associated vessel bearing and vessel-less angiosperms indicating a degree of hydraulic convergence in co-existing plants. Poudyal *et al.*, (2003) found that high  $H_v$  and  $K_s$  value showed highest capacity to supply water to the leaf in Oaks.

The occurrence of cavitation and embolism due to drought would impair the transport of water, especially to the leaves (Li *et al.*, 2009). During periods of drought, due to the lower soil water potential and the high evapotranspiration in the leaves, the probability of embolism is higher, resulting in the death of the plants (Brodribb and Cochard, 2009). According to Choat *et al.*, (2012), drought-induced embolism is recognized as one of the main precursors of the death of woody plants.

Leaf structure character influences leaf energy and water balance. Leaf thickness and mass per unit area (SLM) were higher in early and middle successional species than in late successional species which may have contributed to increase drought tolerance (Abrams and Kubiske, 1990 and Abrams *et al.*, 1994). Longer photosynthetic period probably compensates for the high energy cost of evergreen leaves, with their high SLM (Kikuzawa, 1995). Wilson *et al.*, (1999) reported that high SLM leaves worked better in resource poor environments where retention of captured resource was a higher priority, while low SLM leaves worked best in resource rich environment, and short lived and vulnerable to herbivory. Poudyal *et al.*, (2012b) monitored SLM in *C. indica* and *S. wallichii* and found increased value of SLM when leaves were fully developed and decreased when leaf senescence started with great damages in leaves.

Such increase in SLM as leaves expanded was assigned to the increased thickness of palisade parenchyma by them.

The ability of plants to tolerate water deficits has been frequently attributed to their capacity for osmotic adjustments through accumulation of organic compounds such as amino acids or soluble carbohydrates. Epron and Dreyer (1996) suggested that large increase in soluble carbohydrates contributes in the osmotic adjustment of oaks (*Q. petraea* Matt. Liebl.). They studied four-year old potted saplings of *Quercus petraea* (Matt.) Liebl, which were exposed to water shortage by withholding irrigation. After 10 days, predawn leaf water potential was decreased to -2.0 Mpa and leaf photosynthesis was reduced by 55%. At this stage, starch and sucrose concentrations were decreased by 47% and 48% respectively. Zobel (1996b) used twig starch level as an index of tree stress in forest at Kumaun, India. He concluded that starch in twig near the time of bud break was a useful key of forest health.

Phenological events of the plants are good indicators of climatic differences which manifest very decisively to the environmental factors. Water stress is a precursor inducing phenological activities during summer drought. Hinckely *et al.*, (1979) studied the effect of drought on phenology, growth and physiological processes, as well as the water relations of white Oak (*Quercus alba* L.), and eight other species in oak hickory forest. They found that growth was reduced, die-back increased, net photosynthesis rate depressed to near the compensation point and phenological patterns in the next year were altered.

Phenological development is related to tree water status during leaf expansion (Meinzer *et al.*, 1983). Phenology and seasonal variations in water stress was studied by Reich and Borchert (1984) in numerous trees of twelve species for 1 year, growing at dry and moist sites in a tropical lowland deciduous forest in Guanacaste, Costa Rica. They observed that the seasonal pattern of tree development had a high correlation with seasonal variation in tree water status, but only indirectly with environmental water availability. They observed that, the timing of leaf falls and bud break and, in many species, reversal was determined to a large extent by changes in tree water status. They found no evidence for the control of tree development by seasonal variation in photoperiod or temperature.



Ralhan *et al.*, (1985) studied the phenology of trees in Kumaon Himalayas between 350 and 2150 m a.s.l.. For most species (including all dominants) most of the leaf drop and simultaneous leafing occurred during the warm-dry period of the year.

Rambal and Leterme (1987) observed that *Quercus coccifera* adjusted to water availability along a precipitation gradient mainly by decreasing whole exposed plant leaf area. Wang *et al.*, (1992) presented strong evidences in support of functional linkage of foliar phenology on tree water relations as they observed a significant correlation between the timing of late winter senescence and the hydraulic properties.

Borchert (1994a, b, c) made an extensive research on phenomorphological variations of tropical dry forest trees of Guanacaste, Costa Rica and found that seasonal variation in rainfall controls the phenological activities such as shoot growth, flowering and senescence in individual trees. Leaf shedding, flowering and shoot growth (flushing) were strongly correlated with seasonal changes in tree water status. Further, phenology and water storage capacity of tree species are highly correlated.

Many Neotropical dry forests are characterized by a predominance of trees that lose their leaves for a substantial portion of the year. Low soil moisture and high evaporative demand during the dry season mean that evergreen species in tropical deciduous forests must make substantial structural investments to supply water at high rates to their leaves. Evergreen species have deeper roots, higher leaf mass per area, and greater investment in xylem transport capacity than co-occurring deciduous species (Holbrook *et al.*, 1995). Similarly, Reich (1995) studied the phenology of tropical forests and found that tropical forests may vary in sensitivity to predicted climate change. Phenology of rain forests should change little unless water balance changes markedly and developmental events in rain forests may be relatively insensitive to moderate changes in CO<sub>2</sub> or temperature. Phenology of dry forests could be more sensitive, and in opposite directions, to elevated CO<sub>2</sub> and temperatures. Elevated CO<sub>2</sub> might delay the onset of leaf shedding and stimulate longer life span if stand level transpiration is reduced, whereas higher temperatures could lead to more rapid water depletion, longer leafless periods, and more strongly synchronized phenology.

Williams-Linera (1997) recorded leaf fall, leaf flush, flowering, and fruiting of 107 individuals belonging to twelve deciduous and twelve broadleaved evergreen species and found that foliar phenological patterns varied between deciduous and evergreen species. According to Singh and Kushwaha (2005), duration of deciduousness in tropical trees is related to leafing patterns and resource use rates. Vegetative and flowering phenology of trees in dry tropics is primarily affected by the periodicity of rainfall and soil water availability.

Similarly, Poudyal *et al.*, (2012b) studied that the phenological processes such as leaf drop, leaf emergence, flowering and fruiting occurred in the dry summer months of central Himalayan evergreen tree species. They found that water stress was the precursor including phenological activities in the plant during dry summer.

Poudyal *et al.*, (2012b) studied phenological responses to drought of five evergreens Himalayan trees namely *Castanopsis indica*, *Schima wallichii*, *Rhododendron arboreum*, *Quercus lanata* and *Q. semecarpifolia* and found that reserved starch percentage had a strong effect on phenological activities. It increased during the vegetative bud break and leaf emergence due to accumulation of reserved carbohydrates and decreased during senescence indicating hydrolysis of photosynthates into soluble carbohydrates.

According to Poudyal (2013), *Quercus semecarpifolia* has the longest leaf emergence and expansion period that raised predawn leaf  $\psi_w$  during the period of maximum leaf expansion. Such increase in predawn leaf  $\psi_w$  agrees with Reich and Borchert's (1984) hypothesis that states that phenomorphology varies as a consequences of differences in water availability and changes in internal water status.

Poudyal (2014) studied phenological shifts in response to drought and found that *Castanopsis indica* showed an incessant senescence (November to April) in the third year with low osmotic potentials, in agreement to Nilsen and Muller's (1981) findings; which state that leaf abscission is a morphological response to substantial drought.

Tewari *et al.*, (2016) elucidated that not only temperature but also rainfall periodicity, soil moisture and the related changes of twig  $\psi_w$  in winter and early spring are important drivers of bud expansion and flowering in *Rhododendron arboreum* in central Himalayas. Flower bud size increment was related with increase in twig  $\psi_w$

and soil moisture. Based on their results, they concluded that water availability plays an important role in inducing flowering in *R. arboreum*.

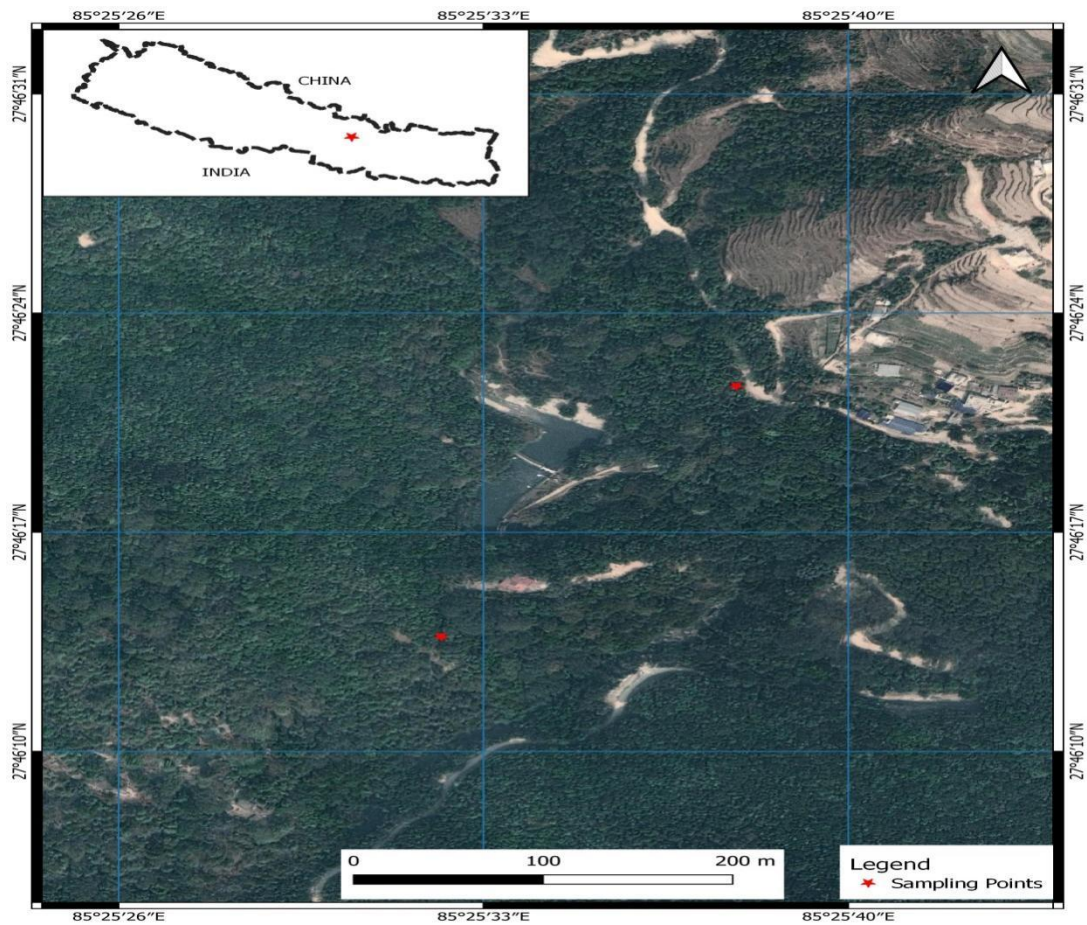
## CHAPTER 3: MATERIAL AND METHODS

### 3.1. Study Area

Shivapuri Nagarjun National Park (SNNP) is located in the country's mid-hills on the northern fringe of the Kathmandu valley and lies about 12 km away from the center of capital city. In 1976, the area was established as a protected watershed & wildlife reserve. In 2002, it was gazetted as Shivapuri National Park, initially covering 144 km<sup>2</sup> (Bhujju *et al.*, 2007) and was extended by the Nagarjun Forest Reserve covering 15 km<sup>2</sup> in 2009 (Pandey, 2010). Geographically Shivapuri forest is located within 27°45' to 27°52' N latitude and 85°16' to 85°45' E longitude. The park covers an area of 144 km<sup>2</sup> stretching about 9km from north-south and about 20 to 24 km east-west. This park claims part of Kathmandu, Nuwakot, Dhading and Sindhupalchowk districts of Nepal. The elevation range is 1350m above sea level to 2732m above sea level. This park is the source of high quality drinking water. The Shivapuri areas provide about 1 million cubic liter of water per day (DNPWC, 2003). It is the watershed of Bagmati, Bishnumati, Nagmati, Syalmati, Sanikhola, Thulikhola and Allekhola. The most important objective of establishing the Shivapuri Nagarjun National Park is to increase the supply of high quality drinking water through the conservation and rehabilitation of the watershed and its flora and fauna. The park area extends over 23 Village Development Committees (VDCs) of three administrative districts of Nepal namely Kathmandu, Nuwakot and Sindhupalchowk of Bagmati Zone.

The study sites were confined at the ranges of 1640 m for *C. tribuloides* and 1655 m for *Q. leucotrichophora* in the south facing slopes. The pre-requisites for choosing the site were:

- Easily accessible
- Natural forest with minimal disturbance
- Adequate number of selected species dominant in the canopy and with desirable size-class for sampling.



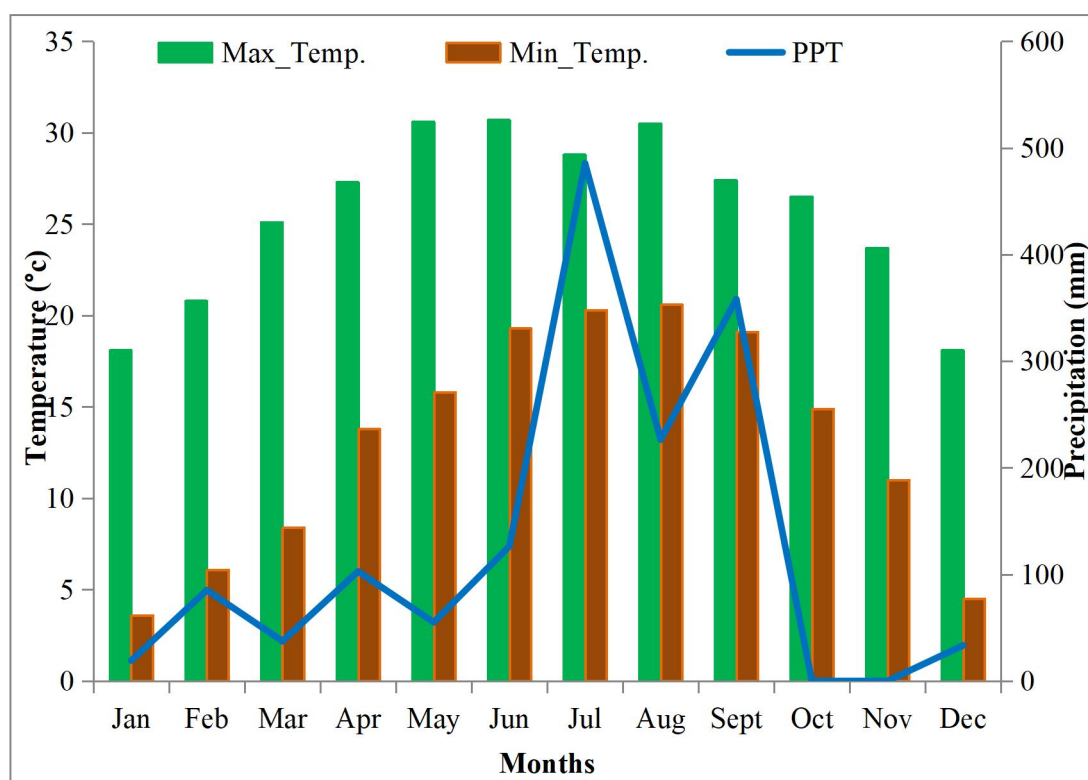
**Figure 1:** Map of the study area

### 3.1.1. Geology and Topography

Geologically, SNNP lies in the Inner Himalayan region. The dominant rocks of the area contain metamorphic rocks such as Gneiss, Phyllite, Limestone and Dolomite which are loamy on the northern aspect and sandy on the southern aspect (Shrestha, 2012). Entire area is characterized by its steep mountainous topography, more than half of the land has slopes greater than 30 degree (Sigdel *et al.*, 2015). Soil erosion and watershed degradation problems are severe in several spots inside the park and surrounding area. Degree of the problems is higher to the northern slopes than the southern slope. Due to dense vegetation and high humus deposits the nutrients in the soil are very high and the runoff rate is relatively slow in the Shivapuri forest but the runoff rate was very fast in the degraded forest (Shrestha, 2015).

### 3.1.2. Climate

The park is located in a transition zone between subtropical and temperate climate. The region has seasonal climate with rainy monsoon seasons (June to September), cool dry winter (October to February) and hot dry summer (March to May). For the study, climatic data were collected from Department of Hydrology and Meteorology, Babarmahal, Kathmandu, Nepal. The maximum rainfall was recorded in July i.e. 485.4 mm and the minimum precipitation of 19.1 mm were recorded in January in 2019. Precipitation was zero during October and November in 2019. In the year 2019, June had maximum average temperature 30.7°C. By observing the data in table, minimum temperature 3.6°C was found in January.



**Figure 2:** Ombrothermic graph for the year 2019.

### 3.1.3. Vegetation

The vegetation of SNNP consists of a variety of natural forest types, depending on altitude and aspects. The dominant vegetation in the park area is *Castanopsis*, *Pinus*, *Quercus* and *Rhododendron* species. The vegetation within the park is broadly divided into two zones, subtropical and temperate zone (Chaudhary, 1998). The Sub-tropical zone consists of *Schima-Castanopsis* and *Pinus roxburghii* forest up to 2000

m asl. The dominant tree species in the *Schima-Castanopsis* forest are *Schima wallichii*, *Castanopsis indica*, *C. tribuloides* and common associates are *Prunus cerasoides*, *Albizia julibrissin*, *Engelhardia spicata*, *Alnus nepalensis* and *Quercus glauca*. *Pinus roxburghi* forest is dominant on southern slope below 1600 m asl. At higher elevation (2000-2732m), mixed temperate broad leaved oak forests are found. *Quercus lanata* is dominant at lower belt (1900-2200m asl.) and *Quercus semecarpifolia* near the summit (2200-2732m asl.). Species like *Rhododendron arboreum*, *Lyonia ovalifolia*, *Myrica esculenta* & *Lindera nacusia* are common associated of oak forests.

#### **3.1.4. Species characteristics**

*Quercus leucotrichophora* A. Camus is an evergreen tree growing up to a height of 15-25m belonging to Fagaceae family commonly known as Banjh oak (Forestry Nepal, 2014). In Nepal, it is commonly found at an altitudinal range of 1500-2700 m in forested areas in association with *Rhododendron arboreum* (Manandhar, 2002), *Lyonia ovalifolia*, *Myrica esculenta* (Thadani and Ashton, 1995). It bears stalked, ovate to lanceolate, acuminate, serrate, leathery, and dark green leaves which are glabrous above and densely white or gray pubescent beneath. It flowers in April to May and fruits in August to October. Male flowers are slender and drooping spikes. Female spikes are sessile and axillary. An acorn is solitary (Manandhar, 2002). The Banjh oak is one of the most useful trees of Himalaya. The leaves are mostly used as a cattle fodder. The timber is used for agricultural implements but is not particularly valuable as it is hard to work. Many types of leaf galls are found in the tree caused by different types of insects. The galls on the leaves are a natural source of gallic acid, a potential anti tumoral agent (Patni *et al.*, 2012).

*Castanopsis tribuloides* (Sm.) A. Dc. is a medium sized evergreen tree that belongs to family Fagaceae growing around 12- 18 m tall. It is found in east, central as well as western Nepal at elevations up to 1800 m in the Himalayas. It is common in sub-tropical forests. The tolerably straight, cylindrical bole can be 60 cm in diameter and free of branches for up to 12 m (tropical.theferns.info, retrieved on 2021-01-09). It is in leaf all year. Flowering occurs from May to June, and the seeds ripen from October to November. The species is monoecious in nature (individual flowers are

either male or female, but both sexes can be found on the same plant) and is pollinated by wind. ([www.pfaf.org](http://www.pfaf.org), retrieved on 2021-01-09).

### **3.1.5. Human activities**

Occasional cutting down of branches of tree species as for fodder & firewood had been observed. Similarly, grazing of domestic animals were the common activities found within studied area. However, tree species were normally undisturbed may be being the protected area.

## **3.2. Methodology**

*Castanopsis tribuloides* (Sm) A. Dc. and *Quercus leucotrichophora* A. Camus were taken as experimental plants. At first six trees, three of each species of *C. tribuloides* and *Q. leucotrichophora* were marked in the study sites for the measurements. Measurements of different water relation parameters such as specific leaf mass (SLM), starch estimation, twig conductance, pressure-volume (P-V) curve analysis, soil moisture content and phenological development such as leaf initiation, leaf maturation, flowering, fruiting, senescence, etc. were estimated. Estimation of reserved starch and phenological observation was done in the field whereas the osmotic potential of tissues, its elasticity and relative water content at zero turgor of collected samples were determined by analyzing the PV- curves in the laboratory. Twig conductance and specific leaf mass (SLM) were also measured in the laboratory using samples collected in the field. For all parameters studied, three replicate plants were taken and measurements were carried out in the marked trees on monthly basis throughout the study which started from 2019/02/ 04 to 2020/01/28.

### **3.2.1. Soil moisture content**

At first, 100 grams of soil samples were collected from the base of selected plants at 15cm and 30 cm depth from the area around 1 m of the plant. Immediately, the soil samples were kept in air tight plastic bag after taking out from the dig. Then the samples were carried to the laboratory and the following procedure was carried out:

- a. At first, the collected soil samples were filtered from impurities like stone, pebbles, etc. About 50 gm of soil was weighed.
- b. Soil samples were kept in a dry oven at temperature of 70-80°C for about 48 hours.



c. The dry weights of soil samples were taken after 48 hours when they were completely dry.

d. Soil moisture content of each sample was measured as follows:

$$\text{Soil moisture content (SMC \%)} = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Dry weight}} \times 100\%$$

### 3.2.2. Pressure Volume Analysis

Pressure volume (P-V) analysis was performed using the pressure chamber by free bench/transpiration technique (Pallardy *et al.*, 1991). P-V curves were used to determine the relationship between water potential and relative water content (RWC) which helped to determine several properties of plant that may affect its water relation.

Curves were analyzed graphically by plotting the inverse of balance pressure (1/BP) over relative water content (RWC) of the foliage or the volume of water expressed. Subject to certain assumptions, the resultant relationship displayed a linear and a non-linear region. A straight edge was used to help judge where the linear region ended and a line was drawn through the points in the linear region. This line represented the inverse osmotic potential at various water contents and the Y-intercept was an estimate of the inverse osmotic potential at full saturation.



**Plate 1:** Pressure Chamber fitted with nitrogen gas cylinder.

### 3.2.3. P-V Theory

The water potential is determined by several components important for cells and their surroundings. Following Gibbs (1931), water potential ( $\psi_w$ ) is given by

$$\psi_w = \psi_s + \psi_p + \psi_m + \psi_g \quad (4.1)$$

where the subscripts s, p, m and g represent the effect of solute, pressure porous, matrices and gravity respectively. These components affect  $\psi_w$  in specific ways. Dissolved solutes lower the chemical potential of water by diluting the water and decreasing the number of water molecules able to move compared to the reference, pure water. In a similar way, matrices that are wet table have surface attractions that decrease the number of water molecules able to move. External pressure above atmospheric increases the ability of water to move but below atmospheric decreases it. Gravity similarly increases or decreases the ability of water to move depending on whether local pressure is increased or decreased by the weight of water.

In the case of cells, the gravitational potential can be ignored because they become significant only at heights greater than 1 m in vertical water columns, as in trees. In this case the equation 4.1 becomes

$$\psi_w = \psi_s + \psi_p + \psi_m \quad (4.2)$$

At its simplest, the cell consists of two compartments: the protoplast or symplast inside and external solution or apoplast outside. The equation (4.2) is then applied to each compartment. The protoplast contains a solution under pressure (turgor) applied by the walls. The protoplast water potential is then

$$\psi_{w(p)} = \psi_{s(p)} + \psi_{p(p)} \quad (4.3)$$

where the subscript (p) denotes the protoplast compartment. The  $\psi_m$  can be ignored because the water content generally is high and there are no air-water interfaces.

The apoplast contains a solution in the porous cell wall subjected to local pressures generated by surface effects of the wall matrix. The apoplast water potential is

$$\psi_{w(a)} = \psi_{s(a)} + \psi_{m(a)} \quad (4.4)$$

where the subscript (a) denotes the apoplast compartment. The  $\psi_p$  can be ignored because the external pressure is atmospheric. For flows commonly present, water potential differences across membranes are so small that a near equilibrium (local equilibrium) exists between the protoplast and its cell wall (Molz and Ferrier, 1982). So it is assumed that

$$\Psi_{w(a)} = \Psi_{w(p)} \quad (4.5)$$

Substituting Equations (4.3) and (4.4) in Equation (4.5) gives:

$$\Psi_{s(a)} + \Psi_{m(a)} = \Psi_{s(p)} + \Psi_{p(p)} \quad (4.6)$$

Pressurization in pressure chamber raises the cell  $\psi_w$  above that of the xylem, and water flows into the xylem. By adjusting the pressure, the flow can be stopped when water just fills the xylem and a flat film appears on the cut surface. This balancing pressure (BP) exactly relieves the tension that had been acting on the xylem solution. The negative of BP thus measures the tension in the apoplast, i.e.  $\Psi_{m(a)}$ .

$$-BP = \Psi_{m(a)} \quad (4.7)$$

Substituting Equation (4.6) in Equation (4.3)

$$\Psi_{w(a)} = \Psi_{s(a)} - BP \quad (4.8)$$

As the pressurization removes water out of the cells, the solution in the walls is diluted and its  $\psi_s$  approaches zero (Boyer, 1995). Thus the equation becomes

$$\Psi_{w(a)} = -BP \quad (4.9)$$

From Equations (4.3), (4.5) and (4.9)

$$-BP = \Psi_{s(p)} + \Psi_{p(p)} \quad (4.10)$$

Thus during PV analysis, the negative of measured BP is the sum value of osmotic potential and turgor pressure of protoplasts.

### 3.2.4. Balance Pressure Measurement

The 'bench-drying' method for pressure-volume (P-V) curve analysis was implemented by using a pressure chamber (PMS Instruments, Corvallis, Oregon) with a limit of 7 MPa and free transpiration technique (Pallardy *et al.*, 1991).

To produce P-V curve, water content and water potential of twigs were measured simultaneously and repeatedly where water content was expressed as RWC and the water potential as the balance pressure (BP) with negative sign.

For drawing P-V curves, leafy twigs were collected from the experimental plants and cut end of twigs were immediately dipped in water for the avoidance of embolism. The samples were covered with plastic bags to reduce the transpiration and kept for rehydration at least for 24 hours that allowed absorbing water under condition of minimum transpiration and water potential became less than 0.3 bars (Zobel, 1996a).

The twig was cut to a size appropriate to pressure chamber. Leaf surface was made dry by soaking with tissue paper, any dead and loose parts were removed, and the bark was stripped from the cut end. The cut end of twig was inserted and then fitted into the cork and weight (twig + cork) was taken and finally BP was measured. Time, mass and BP was recorded. After each reading, the twig was left for drying in the air by hanging in an open area so as to allow free transpiration. After an interval of time (usually 5-10 min in first few readings, then 30 min of interval and finally up to one hour in later readings) the measurements of mass and BP were repeated. The measurement was continued till the leaf completely wilted or no exudation came from the twigs. After the sample was completely dehydrated, it was removed from the cork and the cork was weighed. The twigs were dried at 80° C for about 24 hours and dry weight of the twigs was recorded. For each measurement of BP and fresh weight the relative water content (RWC) was calculated using the formula:

$$\text{RWC (\%)} = \frac{F-D}{S-D} \times 100\%$$

Where,

RWC= Relative Water Content

F = sample + cork weight for each time

S = sample + cork weight for initial measurement (saturation weight).

D = dry weight + cork weight

### 3.3. Components of Plant Water Potential

#### 3.3.1. Osmotic potential ( $\Psi_s$ )

According to Kramer and Boyer (1995),  $\Psi_s$  approximates  $-RT \cdot n/V$ , where R is the gas constant, T is temperature and n/V is the molar concentration of solutes.

Thus,

$$\frac{1}{\Psi_s} = \frac{-V}{nRT}$$

Where, n represents number of mole solutes and V is the volume of cell.

In turgor less tissue,

$$\frac{-1}{BP} = \frac{-V}{nRT}$$

Or,

$$\frac{1}{BP} = \frac{V}{nRT}$$

After pressurization the water moves out from the cell thus affecting V but the number of solutes n remains the same. As T and n are constant, nRT is constant. Thus, graph of 1/BP over V becomes linear. A linear portion in P-V curve represents changes in  $\Psi_s$  along with cell volume. The linear portion and its regression line was used to calculate  $\Psi_s$  at full turgor (at a point where the extrapolation of linear portion meets the abscissa) and at zero turgor (at a point where the curve meets linear).

#### 3.3.2. Relative water content at zero turgor

Relative water content (RWC) was calculated from PV curve. At the point where P-V curve becomes linear (turgor less point), RWC at zero turgor was determined. RWC was calculated using formula:

$$\text{RWC (\%)} = \frac{\text{present weight of sample} - \text{dry weight}}{\text{weight of sample at full saturation} - \text{dry weight}} \times 100\%$$

### 3.3.3. Index of elasticity ( $\epsilon$ )

Elasticity of the cell wall determines the rate of change of turgor pressure of a cell with respect to changes in water. Elastic modulus ( $\epsilon$ ) of a twig can be calculated from P-V relation. The index of elasticity ( $\epsilon$ ) is defined as the change in  $\Psi_p$  for a given fractional change in the weight of symplastic water (Tyree, 1981; Tyree and Jarvis, 1982). To calculate the  $\epsilon$ , all values of  $\Psi_p$  above zero turgor were plotted against RWC; the slope of a linear regression line through these points was used as  $\epsilon$ .

In few samples 'Plateaus' occurred. The 'plateaus' are apparently associated with apoplastic water that is accumulated in the intercellular spaces of leaf near full turgidity, and act to buffer changes in the leaf xylem pressure potential as tissue dehydrates (Parker and Pallardy, 1987). A plateau from either natural or artificial rehydration violates the requirements of constant apoplastic water content and causes an abnormally rapid decrease in RWC relative to that in (Parker and Pallardy, 1987). It is reported that the presence of plateau or 'free water' in the region of high  $\Psi_p$  where tree  $\Psi_w$  remains stable or declines only slightly with tissue dehydration (Bahari *et al.*, 1985). Data sets with plateaus were corrected by assuming that the beginning of the steep decline in water potential ( $\Psi_w$ ) represented full turgor (Zobel, 1996a).

### 3.3.4. Index of desiccation tolerance (IDT)

An index of desiccation (IDT) can estimate the net effect of RWC at turgor loss ( $\text{RWC}_z$ ), osmotic potential at zero turgor ( $\Psi_{sz}$ ) and high tissue elasticity, in which tissue can vary in different ways. IDT was computed using these three tissue properties that conifer desiccation tolerance: low  $\text{RWC}_z$ ,  $\Psi_{sz}$  and high tissue elasticity ( $\epsilon$ ) (Lambers *et al.*, 1998). The lowest end of the range of  $\text{RWC}_z$ ,  $\Psi_{sz}$  and low  $\epsilon$  was assigned a relative value of 100, indicating high tolerance while high end of range was allocated 0 indicating low tolerance. Each parameter was assigned a score (0-100) based on its relationship to maximal and minimal values and hence taken as relative values. The IDT was calculated using mean values of  $\text{RWC}_z$ ,  $\Psi_{sz}$  and  $\epsilon$  as follow:

$$\text{IDT} = \frac{\text{relative } \epsilon + \text{relative } \text{RWC}_z + \text{relative } \Psi_{sz}}{3}$$

### **3.4. Twig Conductance**

Different attributes of twig conductance i.e. Huber value, hydraulic conductance, specific conductance and leaf specific conductance were measured in the sampled trees based on the methods described by Ewers and Cruiziat (1991).

For the measurement of xylem conductance, about 2 years' old twigs were cut from all the experimental plants and dipped immediately in water by its cut end and was carried to the laboratory. About 15 cm of straight portion of stem without leaf was cut under water and bark at the basal end of the twig was removed. Then, it was attached to plastic tube connected with reservoir containing oxalic acid (0.1 M, 1.26 g/l) solution. The segment was allowed to remain vertical till the solution started to drop from the free end (i.e. upper end). Paraffin was used to check the leakage, if any.

For the measurement of flow rate, the twig segment was held horizontally at the measured distance from the reservoir level. A vial containing loosely packed tissue paper was weighed. The rate of flow was calculated taking difference in weight of vial before and after the flow. The process was repeated until nearly constant flow rate was obtained. Leaves beyond the mid-point of the segment were taken and the leaf area was calculated by multiplying the length, breadth and coefficient factor. The sample was then transferred to another tube with 0.1 saffranin solution. The valve was opened and the flow was permitted until both the ends of the twig stained. Then length of twig, upper and lower diameter of twigs as well as upper and lower diameter of pith was measured using vernier caliper, scale and magnifying lens. The percentage of effective xylem was estimated taking thin transverse section of twig at middle point of stem by means of hand lens.



**Plate 2:** Measurement of flow rate of twig using oxalic acid and measurement of staining xylem using saffranin.

Then all the parameters of xylem conductance were calculated using following given formula:

### 3.4.1. Pressure gradient

Pressure gradient is the change of pressure (especially of fluid) per unit distance. It describes in which direction and at what rate pressure is changing around the location.

It was calculated using the formula:

$$\text{Pressure gradient (Pg) (kPa/mm)} = \frac{\text{pressure head(cm)}}{10.2\text{kPa/cm}} \times \frac{1}{\text{length of segment(mm)}}$$

### 3.4.2. Effective xylem area (A<sub>WE</sub>)

The effective xylem area was calculated by using following formula:

$$A_{WE} \text{ (mm}^2\text{)} = \text{Average Xylem Area} * \text{Percentage of Stained Xylem} \dots\dots\dots (1)$$

$$\text{Average Xylem Area (mm}^2\text{)} = \frac{\pi}{8} \times (d_{lw}^2 - d_{lp}^2 + d_{sw}^2 - d_{sp}^2) \dots\dots\dots (2)$$

Where,

$d_{lw}$  = diameter of large end wood



$d_{lp}$  = diameter of large end pith

$d_{sw}$  = diameter of small end wood

$d_{sp}$  = diameter of small end pith

### 3.4.3. Huber Value ( $H_v$ )

Huber value ( $H_v$ ) named after Huber (1928) is defined as the ratio of sapwood area divided by the amount of leaf surface area distal to the stem segment (Ewers, 1985). Sap wood area means wood cross-sectional area.

$$\text{Huber Value } (H_v) = \frac{\text{average xylem area}}{\text{leaf area}}$$

Where, leaf area =  $l \times b \times x$

Here,  $l$  = length of leaves;  $b$  = breadth of leaves and  $x$  = coefficient factor i. e.  $x = 0.654$  (Adhikari, 2000) for *Castanopsis tribuloides*.

### 3.4.4. Hydraulic conductivity ( $K_h$ )

Hydraulic conductivity ( $K_h$ ) is equal to the ratio between water flux through an excised stem segment and to pressure gradient causing the flow (Tyree and Ewers, 1996).

$$K_h (\text{Kg m s}^{-1}\text{MPa}^{-1}) = \frac{\text{flow rate}}{\text{pressure gradient}}$$

### 3.4.5. Specific Conductivity ( $K_s$ )

Specific conductivity ( $K_s$ ) is a measure of the porosity of wood which is equal to  $K_h$  divided by the sapwood cross-sectional area (Tyree and Ewers, 1996).

$$K_s (\text{kg m}^{-1}\text{s}^{-1}\text{MPa}^{-1}) = \frac{\text{hydraulic conductivity}}{\text{effective xylem area}}$$

### 3.4.6. Leaf specific Conductivity ( $K_l$ )

Leaf specific Conductivity ( $K_l$ ) is equal to hydraulic conductivity ( $K_h$ ) divided by leaf area distal to the segment ( $A_l$ ).

$$K_l (\text{kg m}^{-1}\text{s}^{-1}\text{MPa}^{-1}) = \frac{\text{hydraulic conductivity}}{\text{leaf area}}$$

### **3.4.7. Specific Leaf Mass (SLM)**

For the measurement of SLM, healthy leaves were taken from the twigs that were carried to the laboratory. The length and breadth of the leaves were measured. Then, area of the leaves was calculated by multiplying length, breadth and coefficient factor of the leaves. Then the leaves were oven dried at 80° C for at least 24 hours, and dry mass were measured. SLM was calculated using the following formula:

$$\text{SLM} = \frac{\text{leaf dry weight}}{\text{leaf area}}$$

### **3.5. Twig Starch Content**

Starch estimation in 0 and 1 year old twigs was done by using 1% iodine potassium iodide solution following Zobel (1996b). 0-year refers to current year and 1-year refers to last year's twig. Three twig segments of about 5 cm long were collected from each numbered trees of the sampled species in the field. Immediately after that, from each twig 2-3 cm length was cut and split longitudinally through the pith with the help of blade. The split portion was flooded with iodine solution. After 30-45 seconds, it was washed by water. By observing the black color stained in the pith, starch was estimated using a hand lens. By the strength of color stained in the twig, percentage of starch was evaluated.

### **3.6. Phenology**

The phenological observations were done in each marked tree as well as in non-sampled trees of the same species for every month's field visit. Twigs were marked in each tree. In each twig, leaves were selected and marked for the observation of any change. The twigs were further observed for leaf fall, leaf flushing, bud break, shoot elongation, flowering and fruiting. Phenological events were tabulated for each month. In every field visit the length and breadth of leaves were measured and the obtained values were multiplied by coefficient factor to calculate leaf area.

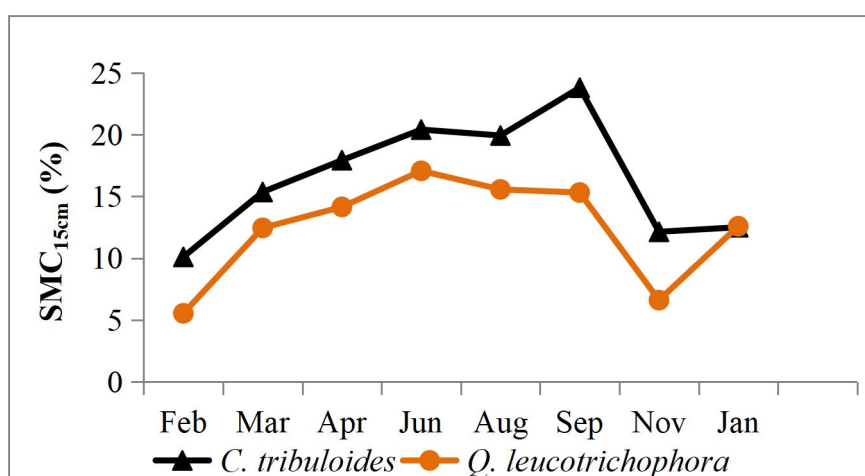
### **3.7. Statistical Analysis**

Mean and Standard error were determined through descriptive statistics in excel (2010). Analysis of variance (ANOVA) - Single factor was done separately for all parameters. Pearson correlation (r) analysis was used to express the relationship between all the studied parameters. SPSS 16.0 for Windows (2010) was used for statistical analysis.

## CHAPTER 4: RESULTS

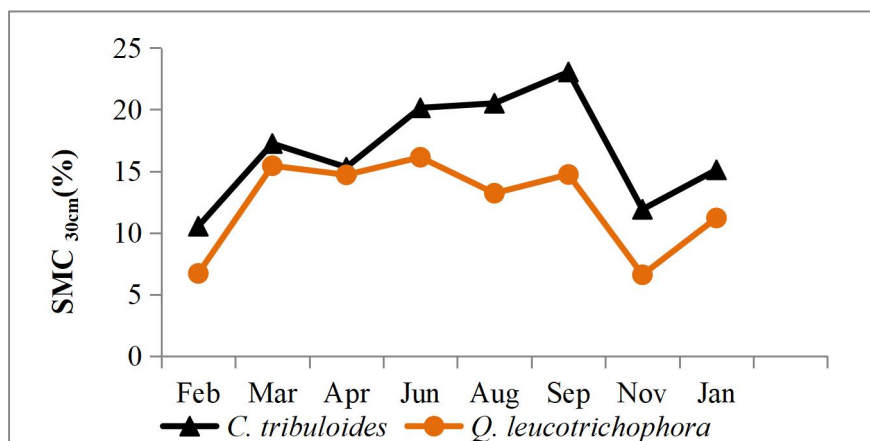
### 4.1. Soil Moisture Content

For both species sampled, soil moisture content at 15cm depth differed significantly among sampling months ( $P < 0.05$ ) (Table: 1) and species ( $P < 0.05$ ) (Table: 2). It was low during summer (February-April) and high in monsoon (June and September) in both species. In early winter (end of November), the values were much lower than that of late monsoon (September). In *C. tribuloides*, moisture percentage ranged from 10.10% (February) to 23.79% (September). *Q. leucotrichophora* showed a variation in soil moisture percentage ranging from 5.53% (February) to 17.06% (June). (Fig: 3)



**Figure 3:** Variation in Soil Moisture Content at 15cm depth (%) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

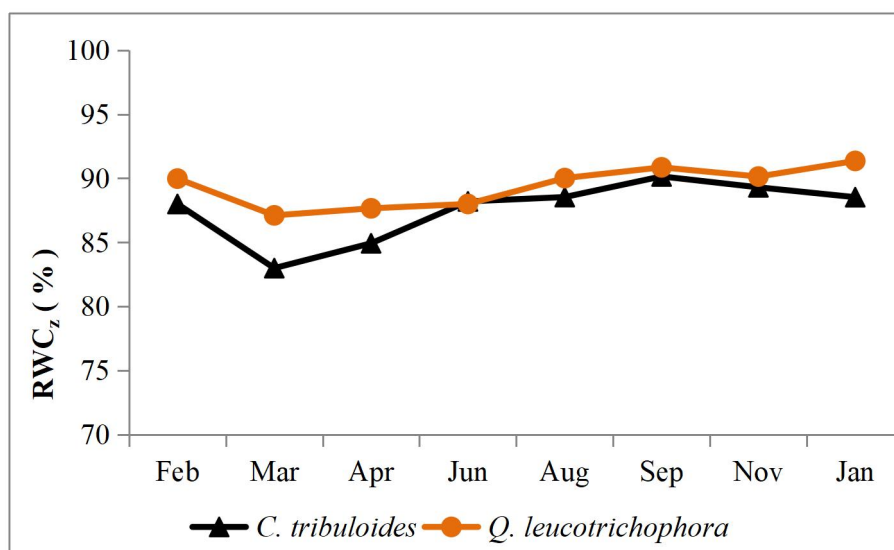
There was significant variation of soil moisture content at 30cm depth with the months sampled ( $P < 0.05$ ) (Table: 1) and species ( $P < 0.05$ ) (Table: 2) but did not show a clear pattern of increase or decrease except for *C. tribuloides* in monsoon period. In *C. tribuloides*, soil moisture percentage was lowest in February (10.53%) and highest in September (23.05%). In *Q. leucotrichophora*, moisture percentage ranged from 6.59% (November) to 16.13% (June). In both species, the values highly decreased from September to November. (Fig: 4)



**Figure 4:** Variation in Soil Moisture Content at 30cm depth (%) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

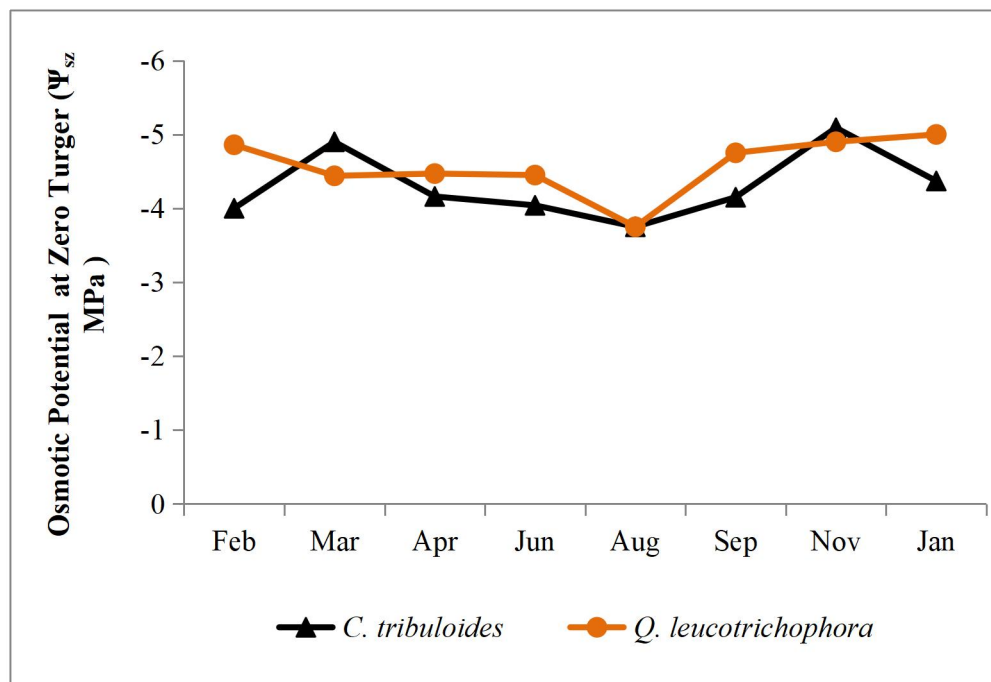
#### 4.2. Pressure Volume Analysis

In both species,  $RWC_z$  declined during the dry months (January to March). An increase was observed from late summer to late monsoon. For both species sampled,  $RWC_z$  exhibited significant variation among sampled months ( $P < 0.05$ ) (Table: 1) and species ( $P < 0.05$ ) (Table: 2).  $RWC_z$  in *C. tribuloides* ranged between 82.99 % (March) to 90.15% (September). In *Q. leucotrichophora*,  $RWC_z$  ranged from 87.11% (March) to 91.36% (September). (Fig: 5)

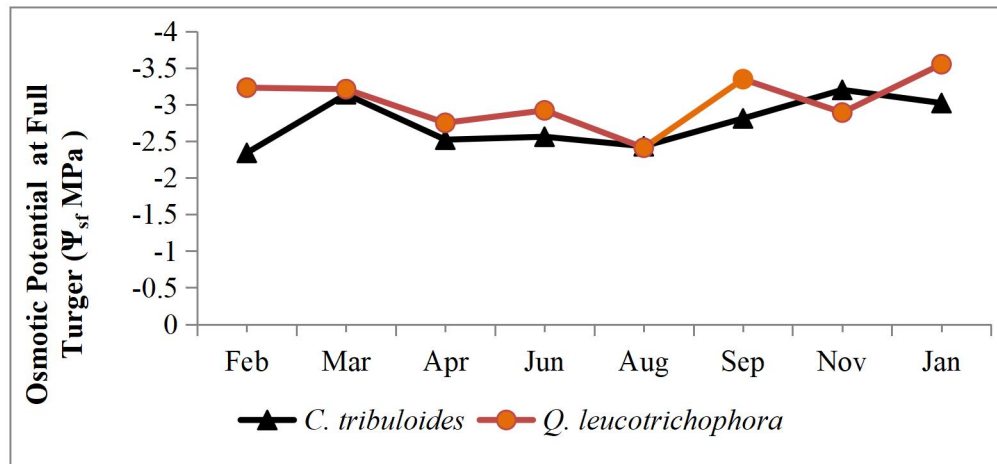


**Figure 5:** Variation in Relative Water Content at zero turgor ( $RWC_z$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

For both species sampled, ANOVA showed that the attributes of water relation parameters ( $\psi_{sz}$  and  $\psi_{sf}$ ) varied significantly among months ( $P < 0.05$ ) (Table: 1) but insignificantly varied among the species ( $P = 0.3$  and  $P = 0.9$ , respectively) (Table: 2). Both  $\psi_{sz}$  and  $\psi_{sf}$  in *C. tribuloides*, increased from summer to monsoon (March to August) and then the values got declined up to November. In *C. tribuloides*, mean  $\psi_{sz}$  was highest (less negative) in August (-3.75MPa) and it was lowest (more negative) in November (-5.09 MPa). Mean  $\psi_{sf}$  was highest in February (-2.34 MPa) whereas it was lowest in November (-3.2 MPa). In case of, *Q. leucotrichophora*,  $\psi_{sz}$  decreased from monsoon to mid -winter (August to January) and then increased up to March while  $\psi_{sf}$  did not show clear pattern of increase or decrease except for summer where the values got declined. Both  $\psi_{sz}$  and  $\psi_{sf}$  were highest in August (-3.75MPa and -2.41MPa respectively) and lowest in January (-5.0 MPa and -3.55 MPa respectively) in *Q. leucotrichophora*. (Fig: 6 and 7)

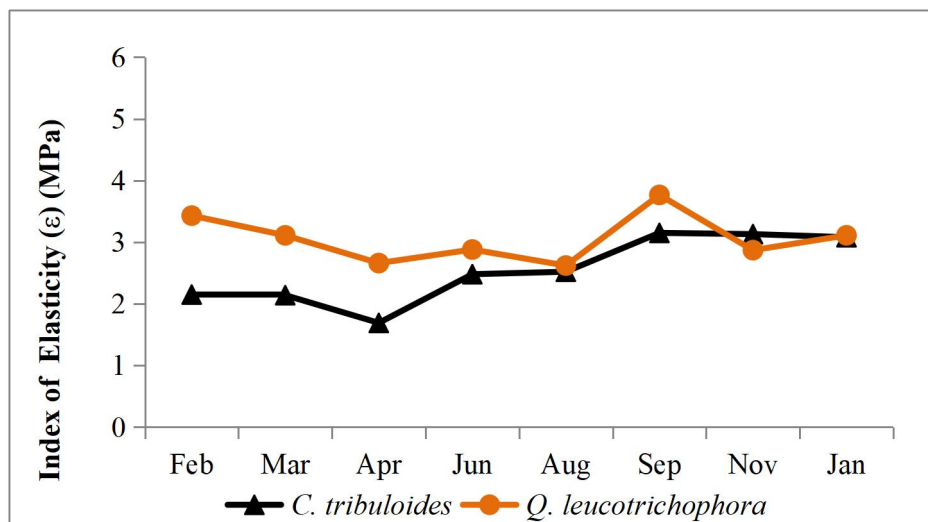


**Figure 6:** Variation in Osmotic potential at zero turgor ( $\Psi_{sz}$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.



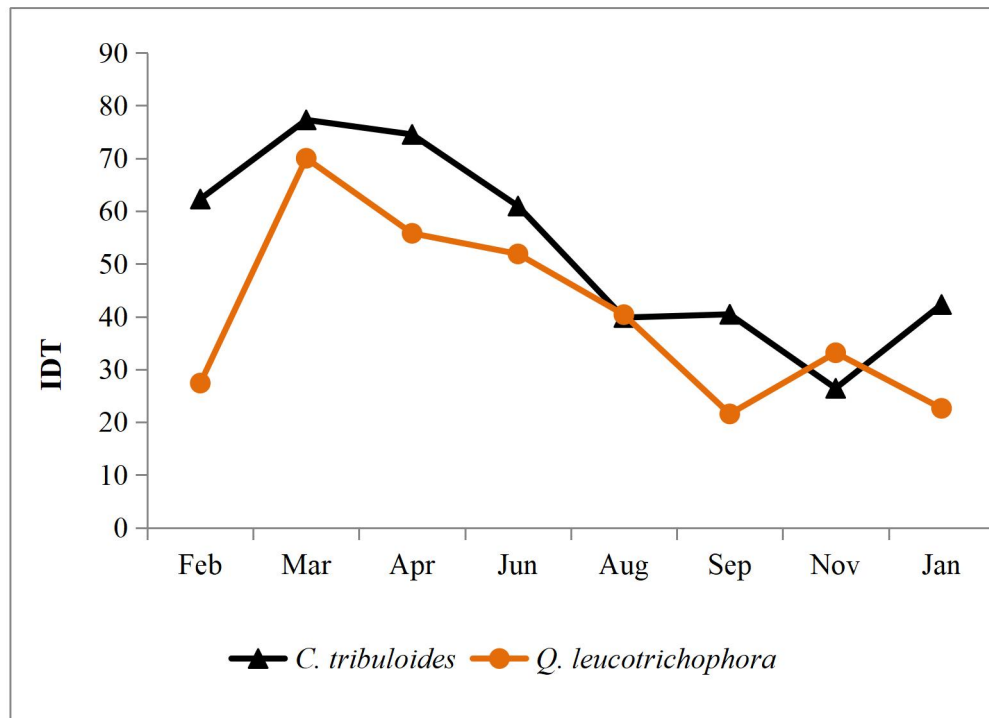
**Figure 7:** Variation in Osmotic potential at full turgor ( $\Psi_{sf}$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

Index of Elasticity ( $\epsilon$ ) showed a significant variation among the studied species ( $P < 0.05$ ) and sampling months ( $P < 0.05$ ) in *C. tribuloides* but insignificantly varied in *Q. leucotrichophora* ( $P = 0.1$ ) (Table: 1 and 2). In both species, index of elasticity decreased during dry months (February-April). In *C. tribuloides*, the highest and lowest value of  $\epsilon$  were recorded in September (3.15 MPa) and April (1.69 MPa) respectively. On the other hand, mean  $\epsilon$  ranged from 2.62 MPa (August) to 3.76 MPa (September) in *Q. leucotrichophora*. (Fig: 8)



**Figure 8:** Variation in Index of Elasticity ( $\epsilon$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

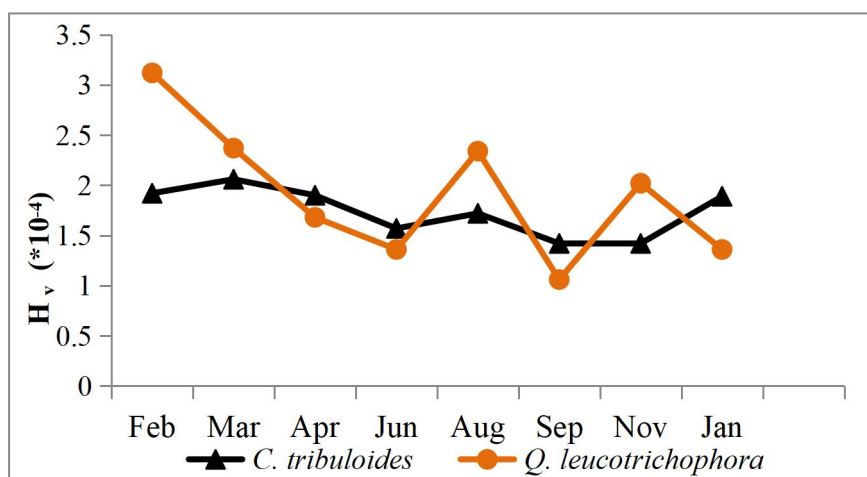
Index of desiccation tolerance (IDT) showed a significant variation with species ( $P < 0.05$ ) (Table: 2) and months ( $P < 0.05$ ) in both species (Table 1). IDT went on decreasing from summer to monsoon (March- August) in *C. tribuloides* while the value decreased from March to September in *Q. leucotrichophora*. IDT ranged from 26.42 (November) to 77.29 (March) in *C. tribuloides*. In *Q. leucotrichophora*, the highest and lowest values of IDT were observed in March (70.02) and September (21.56) respectively. (Fig: 9)



**Figure 9:** Variation in Index of Desiccation Tolerance (IDT) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

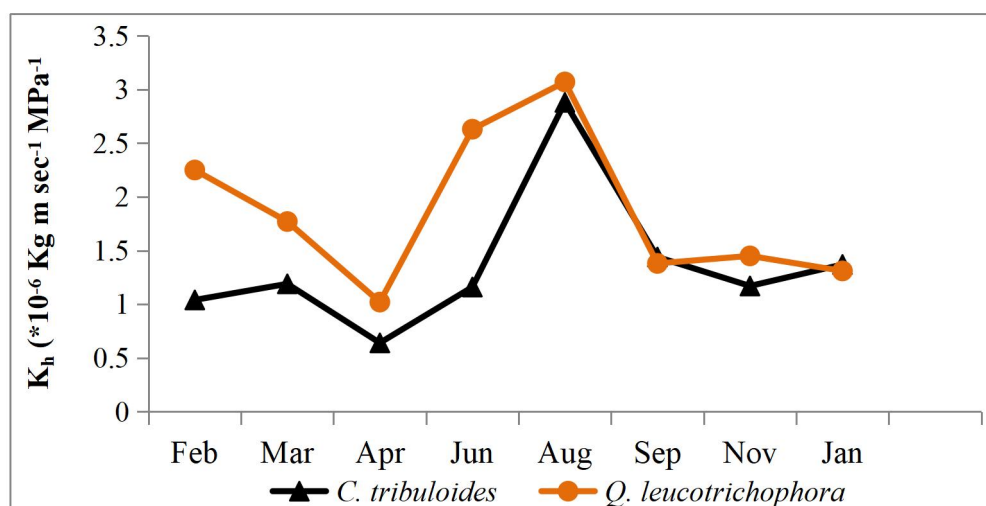
#### 4.3. Twig Conductance

Huber value ( $H_v$ ), which is a proportionality variation between xylem and leaf surface area, varied significantly among sampled months ( $P < 0.05$ ) in both species (Table: 1) but insignificantly varied between two species ( $P = 0.5$ ) (Table: 2). In *C. tribuloides*, a rising trend in  $H_v$  was observed from November to March, being highest in March ( $2.06 \times 10^{-4}$ ) and lowest in September and November ( $1.42 \times 10^{-4}$ ). In *Q. leucotrichophora*,  $H_v$  was observed to be declined from February to June, being highest in February ( $3.12 \times 10^{-4}$ ) and lowest in September ( $1.06 \times 10^{-4}$ ). From August to January the value was fluctuated. (Fig: 10)



**Figure 10:** Variation in Huber value ( $H_v$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*

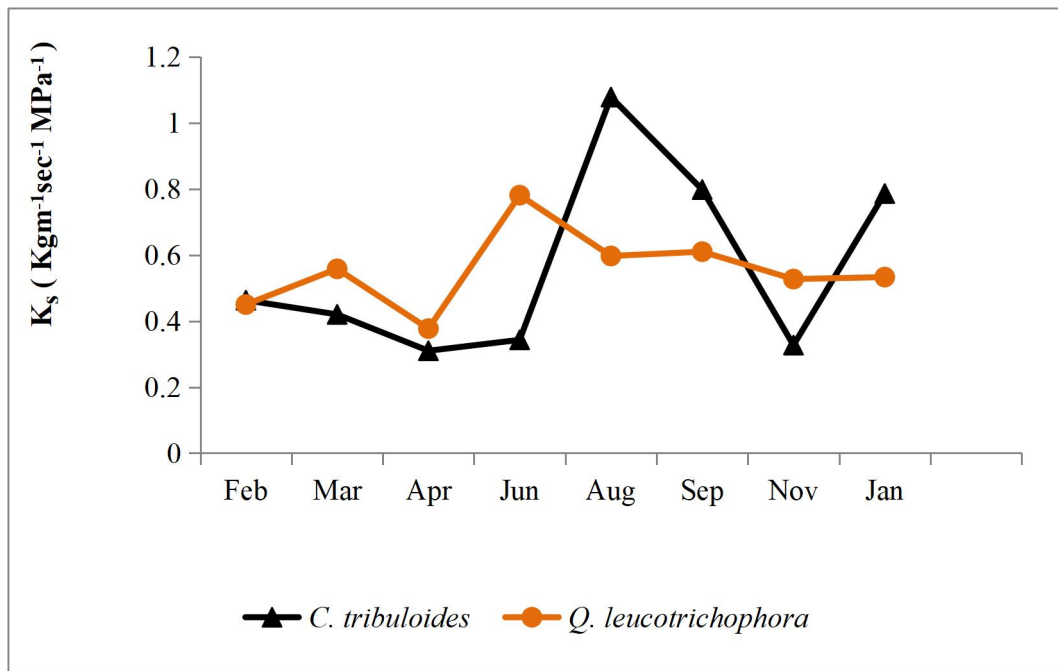
ANOVA showed that the hydraulic conductivity ( $K_h$ ) varied significantly among the sampled months ( $P < 0.05$ ) in both species (Table: 1) but insignificantly differed between two species ( $P = 0.17$ ) (Table: 2). In *Q. leucotrichophora*, hydraulic conductivity ( $K_h$ ) decreased during dry months. An increase was observed from April to August in both species. In *C. tribuloides*,  $K_h$  ranged from  $0.64 \times 10^{-6} \text{ kg m sec}^{-1} \text{ MPa}^{-1}$  (April) to  $2.88 \times 10^{-6} \text{ kg m sec}^{-1} \text{ MPa}^{-1}$  (August). In *Q. leucotrichophora*,  $K_h$  ranged from  $1.02 \times 10^{-6} \text{ kg m sec}^{-1} \text{ MPa}^{-1}$  (April) to  $3.07 \times 10^{-6} \text{ kg m sec}^{-1} \text{ MPa}^{-1}$  (August). (Fig: 11)



**Figure 11:** Variation in in Hydraulic conductivity ( $K_h$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

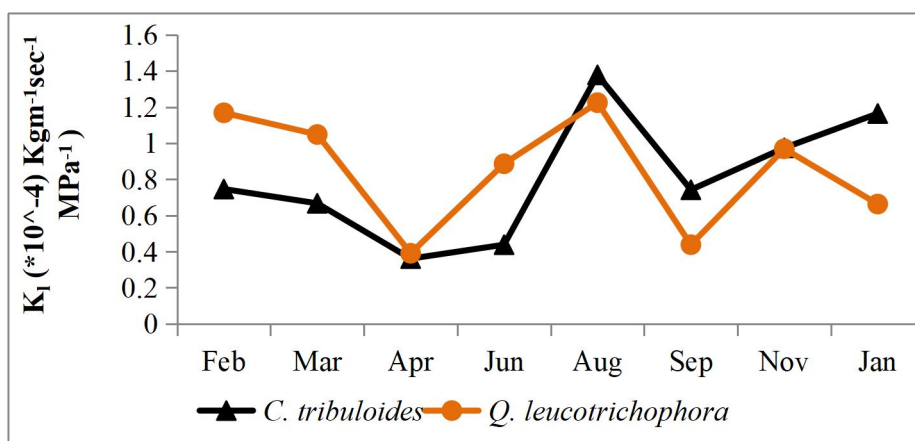


ANOVA showed that the specific conductivity ( $K_s$ ) showed significant variation among the sampled months ( $P < 0.05$ ) in both species (Table: 1) but not with the sampled species ( $P = 0.88$ ) (Table: 2). Specific conductivity decreased from January to April and then increased up to August in *C. tribuloides* while in case of *Q. leucotrichophora*, it did not show clear pattern of change. In *C. tribuloides*,  $K_s$  ranged from  $0.31 \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  (April) to  $1.08 \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  (August). In *Q. leucotrichophora*,  $K_s$  ranged from  $0.38 \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  (April) to  $0.78 \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  (June). (Fig: 12)



**Figure 12:** Variation in Specific conductivity ( $K_s$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

ANOVA showed that the leaf specific conductivity ( $K_l$ ) varied significantly among the sampled months ( $P < 0.05$ ) in both species (Table: 1) but not with the sampled species ( $P = 0.94$ ) (Table: 2). In both species, leaf specific conductivity decreased during dry months and the values increased when monsoon proceeded. In *C. tribuloides*,  $K_l$  was recorded highest during August ( $1.3 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) and lowest during April ( $0.36 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ). In *Q. leucotrichophora*,  $K_l$  ranged highest in August ( $1.22 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) and lowest in April ( $0.39 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ). (Fig: 13)

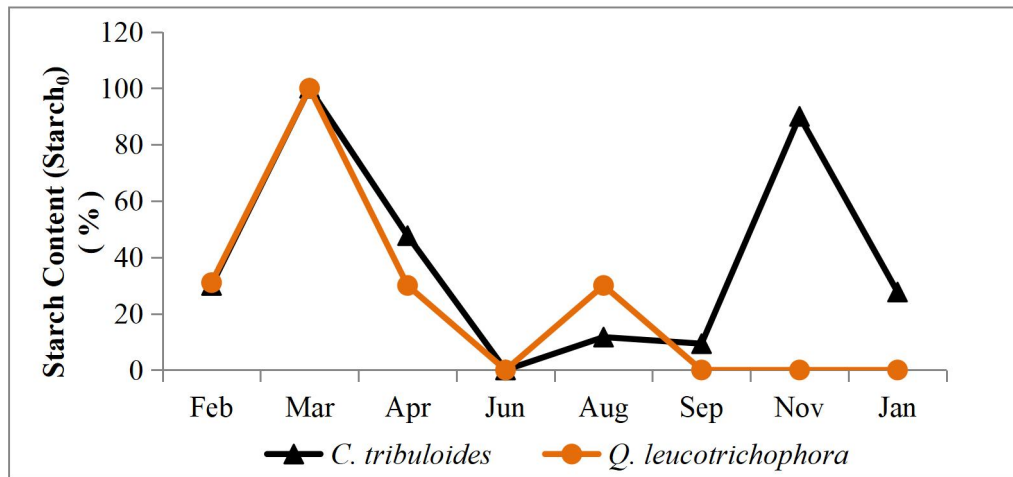


**Figure 13:** Variation in Leaf specific conductivity ( $K_l$ ) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

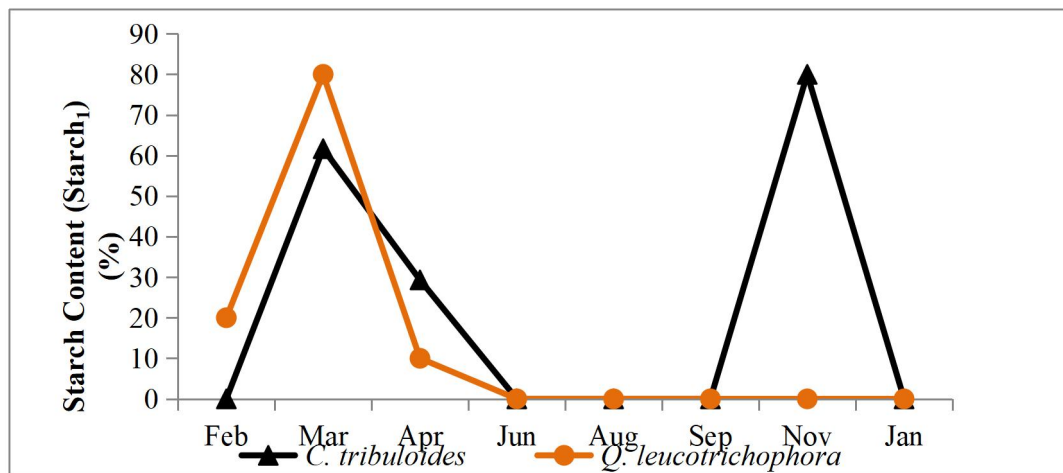
#### 4.4. Twig Starch Content

ANOVA analysis showed a significant variation in starch content in zero-year twig ( $\text{Starch}_0$ ) ( $P < 0.05$ ) but insignificant variation in one-year-twig ( $\text{Starch}_1$ ) ( $P = 0.16$ ) among sampled months (Table: 1) in *C. tribuloides*. However, in case of *Q. leucotrichophora*, insignificant variation was observed in zero-year twig and one year twig among sampled months ( $P = 0.13$  and  $P = 0.36$  respectively) (Table: 1). Also there was insignificant variation in starch content in zero-year twig and one-year-twig among the sampled species ( $P = 0.1$  and  $P = 0.37$  respectively). (Table: 2)

In *C. tribuloides*,  $\text{Starch}_0$  was high in March and November while lower values were found in monsoon period (June- September).  $\text{Starch}_1$  was high in March and November while there was no starch in winter (January and February) and monsoon period (June-September).  $\text{Starch}_0$  in *C. tribuloides*, ranged from 0% (June) to 100% (March) and  $\text{starch}_1$  ranged from 0% (February, June, August, September and January) to 80% (November). In *Q. leucotrichophora*, starch content was high in March and decreased gradually in late summer and early monsoon. There was no starch during post monsoon period. In *Q. leucotrichophora*, starch content of current year ( $\text{starch}_0$ ) was highest in March (100%) and lowest in June, September, November and January (0%). The previous year twig ( $\text{starch}_1$ ) was highest in March (80%) and lowest in June, August, September, November and January (0%). (Fig: 14 and 15)



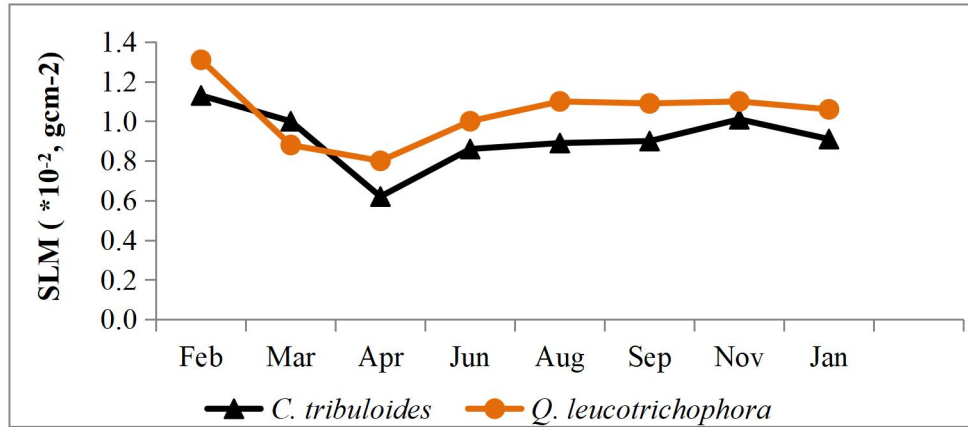
**Figure 14:** Variation in Starch percentage in zero year twig (Starch<sub>0</sub>) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.



**Figure 15:** Variation in Starch percentage in one year twig (Starch<sub>1</sub>) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

#### 4.5. Specific Leaf Mass

For both species sampled, specific leaf mass (SLM) showed significant variation with sampled months ( $P < 0.05$ ) (Table: 1) and species ( $P < 0.05$ ) (Table: 2). Mean value showed significant difference among the sampled species. In both species, SLM decreased in summer (February to April) and increased in monsoon period. In *C. tribuloides*, high and low SLM were observed in the month of February ( $1.13 \times 10^{-2} \text{ g cm}^{-2}$ ) and April ( $0.62 \times 10^{-2} \text{ g cm}^{-2}$ ) respectively. *Q. leucotrichophora*, had the highest SLM in February ( $1.31 \times 10^{-2} \text{ g cm}^{-2}$ ) and lowest in April ( $0.8 \times 10^{-2} \text{ g cm}^{-2}$ ). (Fig: 16)



**Figure 16:** Variation in Specific Leaf Mass (SLM) during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

**Table 1:** ANOVA result showing variation among sampling months in *C. tribuloides* and *Q. leucotrichophora*. Significance values:  $P < 0.05$ .

Parameters	<i>P</i> - value ( <i>C. tribuloides</i> )	<i>P</i> - value ( <i>Q. leucotrichophora</i> )
SMC <sub>15cm</sub>	< 0.0001	< 0.001
SMC <sub>30cm</sub>	< 0.0001	< 0.001
RWC <sub>z</sub>	< 0.0001	< 0.0001
$\psi_{sz}$	< 0.0001	< 0.0001
$\psi_{sf}$	< 0.0001	< 0.0001
$\epsilon$	0.044	0.12
IDT	< 0.0001	< 0.0001
Starch <sub>0</sub>	0.018	0.134
Starch <sub>1</sub>	0.165	0.363
SLM	< 0.001	< 0.001
H <sub>v</sub>	< 0.001	< 0.001
K <sub>h</sub>	< 0.001	< 0.001
K <sub>s</sub>	< 0.001	< 0.001
K <sub>l</sub>	< 0.001	< 0.001

**Table 2:** ANOVA result showing variation between *C. tribuloides* and *Q. leucotrichophora*. Significance values:  $P < 0.05$ .

Parameters	P- value
SMC <sub>15cm</sub>	0.016
SMC <sub>30cm</sub>	0.004
RWC <sub>z</sub>	0.019
$\psi_{sz}$	0.325
$\psi_{sf}$	0.993
$\varepsilon$	0.027
IDT	0.014
Starch <sub>0</sub>	0.107
Starch <sub>1</sub>	0.371
SLM	0.020
H <sub>v</sub>	0.576
K <sub>h</sub>	0.883
K <sub>s</sub>	0.949
K <sub>l</sub>	0.170

#### 4.6. Relationship among the parameters

##### 4.6.1. Correlation of soil moisture content with other variables

Soil moisture content at 15cm depth (%) correlated positively with Soil moisture content at 30cm depth (%), K<sub>h</sub> and K<sub>s</sub>, whereas it showed negative correlation with H<sub>v</sub>, SLM, starch<sub>0</sub> and starch<sub>1</sub> in *C. tribuloides*. Most of the parameters studied like RWC<sub>z</sub>,  $\psi_{sz}$ ,  $\psi_{sf}$ ,  $\varepsilon$ , IDT and K<sub>l</sub> showed insignificant correlation with soil moisture content at 15cm depth. (Table: 3)

In, *Q. leucotrichophora*, it exhibited positive correlation with SMC at 30cm depth (%),  $\psi_{sz}$ , IDT and K<sub>s</sub> and correlated negatively with SLM, H<sub>v</sub> and K<sub>l</sub>. There was insignificant correlation between moisture content and RWC<sub>z</sub>,  $\varepsilon$ , starch<sub>0</sub>. (Table: 4)

Soil moisture content at 30cm depth (%) correlated positively with  $K_h$  and  $K_s$  whereas it showed negative correlation with  $H_v$ , SLM, starch<sub>0</sub> and starch<sub>1</sub> in *C. tribuloides*. Most of the parameters studied like  $RWC_z$ ,  $\psi_{sz}$ ,  $\psi_{sf}$ ,  $\epsilon$ , IDT and  $K_l$  showed insignificant correlation with soil moisture content at 30cm depth. (Table: 3)

In, *Q. leucotrichophora*, it exhibited positive correlation with  $\psi_{sz}$ ,  $\psi_{sf}$ , IDT and  $K_s$  and showed negative correlation with  $RWC_z$ , SLM,  $H_v$  and  $K_l$ . There was no significant correlation with  $\epsilon$ , starch<sub>0</sub> and starch<sub>1</sub>. (Table: 4)

#### **4.6.2. Correlation of $RWC_z$ , $\psi_{sz}$ and $\psi_{sf}$ with other variables**

In *C. tribuloides*, relative water content at zero turgor ( $RWC_z$ ) correlated positively with  $\epsilon$ ,  $K_h$ ,  $K_s$  and  $K_l$  and correlated negatively with  $H_v$ ,  $\psi_{sz}$ ,  $\psi_{sf}$ , IDT, starch<sub>0</sub> and starch<sub>1</sub>. A significant negative correlation of  $RWC_z$  with IDT and  $H_v$  was observed. A significant positive correlation of  $\psi_{sz}$  with  $\psi_{sf}$  and IDT was found.  $\psi_{sz}$  showed positive correlation with  $H_v$  and negatively correlated with  $K_h$ ,  $K_s$  and  $K_l$ .  $\psi_{sf}$  showed positive correlation with parameters such as  $H_v$ , IDT and correlated negatively with  $\epsilon$ ,  $K_h$ ,  $K_s$  and  $K_l$ . (Table: 3)

A significant negative correlation of  $RWC_z$  with  $\psi_{sz}$ ,  $\psi_{sf}$  and IDT and significant positive correlation with  $\epsilon$  was found in *Q. leucotrichophora*.  $RWC_z$  correlated negatively with starch<sub>0</sub> and starch<sub>1</sub> and positively with SLM. In *Q. leucotrichophora*,  $\psi_{sz}$  showed significant positive correlation with  $\psi_{sf}$ , IDT, starch<sub>0</sub> and starch<sub>1</sub> while negative correlation occurred between  $\psi_{sz}$  and  $\epsilon$  and SLM.  $\psi_{sf}$  showed negative correlation with  $\epsilon$  and SLM while correlated positively with IDT, starch<sub>0</sub> and starch<sub>1</sub>. (Table: 4)

#### **4.6.3. Correlation of Index of elasticity with other variables**

In *C. tribuloides*, Index of elasticity correlated negatively with IDT and  $H_v$ .  $\epsilon$  correlated positively with SLM and  $K_l$  (Table: 3). A significant positive correlation of  $\epsilon$  with SLM and a significant negative correlation with IDT were found in *Q. leucotrichophora*.  $\epsilon$  correlated negatively with starch<sub>0</sub> and starch<sub>1</sub>. (Table: 4)

#### **4.6.4. Correlation of IDT**

IDT showed a significant positive correlation with  $H_v$  and negative correlation with  $K_h$ ,  $K_s$  and  $K_l$  in *C. tribuloides* (Table: 3). IDT in *Q. leucotrichophora* correlated positively with twig starch content and negatively with SLM (Table: 4).

#### **4.6.5. Correlation of starch percentage content in twigs**

Both variables of starch (starch<sub>0</sub> and starch<sub>1</sub>) correlated significantly with each other in both sampled species (Table: 3 and 4). Both starch<sub>0</sub> and starch<sub>1</sub> correlated negatively with K<sub>h</sub> and K<sub>s</sub> in *C. tribuloides* (Table: 3). In *Q. leucotrichophora*, starch<sub>0</sub> showed positive correlation with H<sub>v</sub> and K<sub>l</sub> and negatively correlated with SLM. Starch<sub>1</sub> correlated positively with H<sub>v</sub> and negatively with SLM. (Table: 4)

#### **4.6.6. Correlation of SLM**

SLM in *C. tribuloides* correlated positively with K<sub>l</sub> (Table: 3) and with H<sub>v</sub>, K<sub>h</sub> and K<sub>l</sub> in *Q. leucotrichophora* (Table: 4)

#### **4.6.7. Correlation of Huber value**

In *C. tribuloides*, H<sub>v</sub> showed insignificant correlation with K<sub>h</sub>, K<sub>l</sub> and K<sub>s</sub> (Table: 3) while in *Q. leucotrichophora*, H<sub>v</sub> correlated positively with K<sub>h</sub> and K<sub>l</sub> and negatively with K<sub>s</sub> (Table: 4).

#### **4.6.8. Correlation of Hydraulic conductivity**

Hydraulic conductivity showed significant correlation with K<sub>l</sub> and K<sub>s</sub> in both species (Table: 3 and 4).

#### **4.6.9. Correlation between Specific conductivity and Leaf specific conductivity**

Specific conductivity and Leaf specific conductivity correlated significantly with each other in *C. tribuloides* (Table: 3) but insignificantly correlated in *Q. leucotrichophora* (Table: 4).

**Table 3:** Pearson coefficient of correlation between various parameters shown by *C. tribuloides*. Significance values: \*=P < 0.05, \*\*=P < 0.01

	SMC <sub>1</sub> 5cm	SMC <sub>3</sub> 0cm	RW C <sub>z</sub>	Ψ <sub>sz</sub>	Ψ <sub>sf</sub>	ε	IDT	Star ch <sub>0</sub>	Star ch <sub>1</sub>	SL M	H <sub>v</sub>	K <sub>s</sub>	K <sub>l</sub>	K <sub>h</sub>
SMC <sub>1</sub> 5cm		0.94**	0.15	0.09	-0.05	-0.03	-0.01	-0.51	-0.35	-0.55	-0.44	0.34	-0.19	0.32
SMC <sub>3</sub> 0cm			0.15	0.12	-0.13	0.12	-0.06	-0.51	-0.40	-0.38	-0.34	0.50	0.00	0.47
RWC <sub>z</sub>				-0.55	-0.60	0.54	-0.85**	-0.57	-0.41	0.21	-0.77*	0.43	0.45	0.35
Ψ <sub>sz</sub>					0.90**	-0.25	0.83*	-0.05	-0.23	-0.01	0.52	-0.33	-0.08	0.52
Ψ <sub>sf</sub>						-0.52	0.90**	0.03	-0.12	0.02	0.53	-0.53	0.78*	0.59
ε							-0.67	0.10	0.17	0.39	-0.54	0.17	0.34	0.04
IDT								0.17	-0.01	-0.24	0.72*	-0.48	-0.71	0.48
Star ch <sub>0</sub>									0.94**	0.23	0.28	-0.50	-0.08	0.35
Star ch <sub>1</sub>										0.12	-0.02	-0.55	0.11	0.31
SLM											0.03	0.05	0.36	0.12
H <sub>v</sub>												-0.08	-0.08	0.16
K <sub>s</sub>													0.78*	0.86**
K <sub>l</sub>														0.8



															0**
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**Table 4:** Pearson coefficient of correlation between various parameters shown by *Q. leucotrichophora*. Significance values: \*=P < 0.05, \*\*=P < 0.01.

	SMC <sub>1</sub> 5cm	SMC <sub>3</sub> 0cm	RW C <sub>z</sub>	Ψ <sub>sz</sub>	Ψ <sub>sf</sub>	ε	ID T	Star ch <sub>0</sub>	Star ch <sub>1</sub>	SL M	H <sub>v</sub>	K <sub>s</sub>	K <sub>l</sub>	K <sub>h</sub>
SMC <sub>1</sub> 5cm		0.92**	- 0.24	0.4 4	0.0 0	- 0.0 7	0.3 0	- 0.06	- 0.14	- 0.5 4	- 0. 65	0. 60	- 0.3 8	0.2 0
SMC <sub>3</sub> 0cm			- 0.53	0.6 9	0.3 1	- 0.2 2	0.5 6	0.25	0.21	- 0.7 1	- 0. 53	0. 50	- 0.3 8	0.0 7
RWC z				- 0.8 4**	- 0.9 1**	0.7 4*	- 0.9 6**	- 0.64	- 0.61	0.6 94	- 0. 16	- 0. 05	- 0.0 5	- 0.0 7
Ψ <sub>sz</sub>					0.7 9*	- 0.5 6	0.9 1**	0.81 *	0.73 *	- 0.6 3	0. 16	0. 23	0.2 0	0.2 6
Ψ <sub>sf</sub>						- 0.7 7*	0.9 1**	0.69	0.69	- 0.6 4	0. 24	- 0. 08	0.1 4	- 0.0 6
ε							- 0.8 2*	- 0.45	- 0.41	0.7 2*	- 0. 10	0. 13	- 0.1 1	0.0 8
IDT								0.73 *	0.67	- 0.7 5*	0. 16	0. 08	0.1 4	0.1 2
Star h <sub>0</sub>									0.95 **	- 0.3 6	0. 54	- 0. 21	0.3 6	0.0 8
Star h <sub>1</sub>										- 0.3 3	0. 44	- 0. 13	0.2 9	- 0.0 5
SLM											0. 42	0. 05	0.4 8	0.3 8
H <sub>v</sub>												- 0.	0.7 8*	0.4 0

												40		
K <sub>s</sub>													0.1 7	0.5 7
K <sub>l</sub>														0.7 6*

#### 4.7. Phenology

The phenological events (bud break, leaf emergence, flowering & senescence) commenced mostly from the late winter months to late summer (Feb-May) in *Q. leucotrichophora* (Fig: 17) while flowering delayed to September in *C. tribuloides* (Fig: 18). The studied species showed evergreen types of leaf exchange pattern where plants were not naked due to simultaneous leafing. The senescence was confirmed when approximately 25% of the leaves were shed off from the sampled as well as nearby trees.

In *C. tribuloides*, winter buds burst in early March. Then new leaf formation, enlargement and shoot elongation continued until late June. Again, leaf bud emergence occurred in August and leafing in some twigs was observed in November. January to April was found to be the period of leaf drop in *C. tribuloides*. In this species, flowering took place in September. Fruiting started from late December and matured in February. The damages occurred as necrotic spots and holes throughout the leaves. (Fig: 18)

In *Q. leucotrichophora*, bud break occurred in early March followed by leaf initiation and twig emergence. Leaf enlargement continued till June. Senescence was rapid in this species and sometimes even one-month-old leaf dried and fell off. High leaf drop was observed in March and April. Second flushing took after the rainy season in this species. In *Q. leucotrichophora*, 90% shoot elongation was completed during rainy season (August-September). Flowers appeared during March-May. Visible fruits occurred in June and matured in November to January. Leaves were found damaged by insects, necrotic patches, bulges and holes. (Fig: 17)

Leaf area showed significant variation among the sampled months and both sampled species ( $P < 0.05$ ). Mean leaf area showed the highest and lowest value in March (468.33 mm<sup>2</sup>) and June (336.72 mm<sup>2</sup>) respectively in *C. tribuloides*. In *Q.*

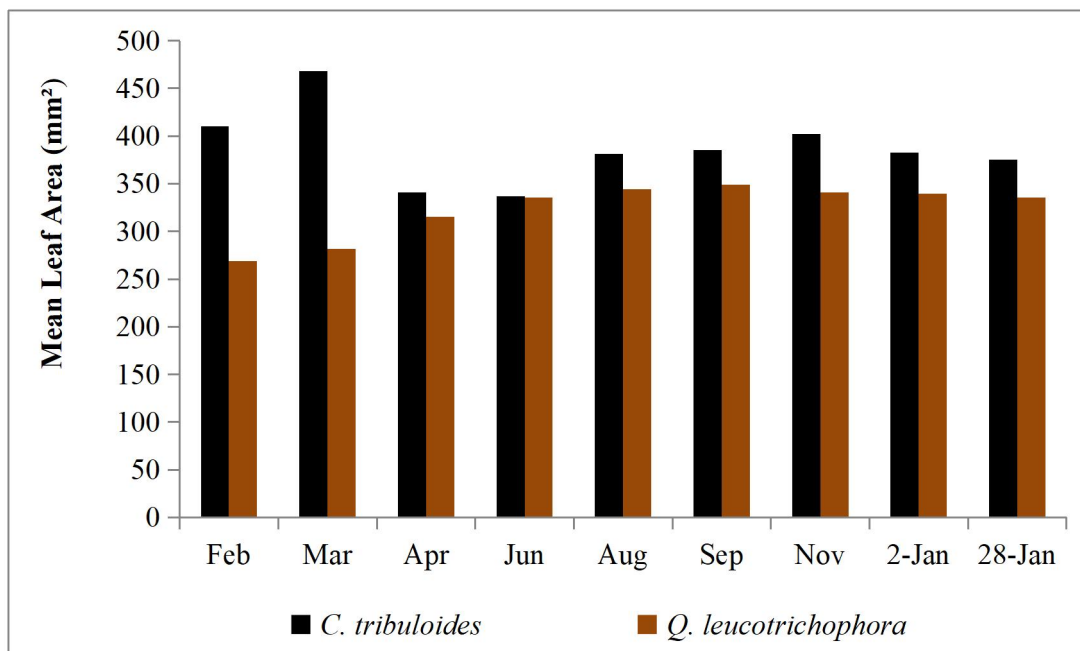
*leucotrichophora*, it exhibited highest value in September (348.64 mm<sup>2</sup>) and lowest during February (268.91 mm<sup>2</sup>). (Fig: 19)

Phenophases	Months									
	Feb	Mar	Apr	June	Aug	Sep	Nov	Jan2	Jan28	
Flowering		Red								
Fruiting				Yellow						
Leaf damage	Brown						Brown			
Leaf senescence	Green									Green
Leaf emergence		Light Green						Light Green		

**Figure 17:** Phenological activities in *Q. leucotrichophora* during all sampling months.

Phenophases	Months									
	Feb	Mar	Apr	June	Aug	Sep	Nov	Jan2	Jan28	
Flowering						Red				
Fruiting	Yellow							Yellow		
Leaf damage	Brown						Brown			
Leaf senescence	Green									Green
Leaf emergence		Light Green						Light Green		

**Figure 18:** Phenological activities in *C. tribuloides* during all sampling months.



**Figure 19:** Variation in leaf area during February 2019 to January 2020 in *C. tribuloides* and *Q. leucotrichophora*.

## CHAPTER 5: DISCUSSION

The present study exhibited monthly variation in water relation parameters and phenological behaviors in *C. tribuloides* and *Q. leucotrichophora*. The accessibility of soil water to plants depends primarily on amount of water stored in the soil and its relation to soil moisture content (Lambers *et al.*, 1998). In both species soil moisture percentage in both depth i.e. 15cm and 30cm were highest in September when soils were wet after three months of precipitation similar to the findings of Adhikari (2000). The minimum soil moisture content was observed during late winter (February) in *C. tribuloides*. In *Q. leucotrichophora*, moisture content reached to its minimum value in November when no notable rainfall occurred. During this dry period, due to correspondingly increased surface temperature which ultimately increased surface evaporation and the decline of soil moisture reserve for plants in the absence of significant rainfall. Soil moisture content at 30cm depth correlated significantly with soil moisture percentage at 15cm depth for both species and specified the same seasonal patterns of variation. Variation in soil moisture content might be due to the differences in rooting depth, the length of growing period, capacity to extract water, etc.

*Q. leucotrichophora* showed significant correlation between  $\psi_{sz}$  and soil moisture content at both depth 15cm and 30cm indicating its ability to accumulate solute concentrations during low water availability while in *C. tribuloides*, there was insignificant correlation. Poudyal (2004) reported a significant correlation between mean soil  $\psi$  and  $\psi_{sz}$  in *Quercus* species and some other Himalayan trees while Abrams (1990) found no correlation between predawn leaf  $\psi$  and  $\psi_{sz}$  for several oak species both under field and green house conditions. When soil moisture content decreases in dry months, living cells adjust their water status by accumulating osmotically active compounds, which lower the osmotic potential and hence maintain turgor (Lambers *et al.*, 1998).

Osmotic adjustment is one of the common adaptation strategies exhibited by plants to tolerate low water potential (Abrams, 1990). Osmotic potentials at both zero and full turgor in studied species decreased after monsoon as the dry periods proceeded. A significant difference in  $\psi_{sz}$ ,  $\psi_{sf}$ ,  $\epsilon$  and  $RWC_z$  might be attributed to the internal recycling of solutes during the dry periods and to timing of phenological activity.

Such type of observation has been reported by Poudyal (2004) in some Himalayan trees at Phulchowki hill. *Q. leucotrichophora* had the lowest osmotic potential values (both  $\psi_{sf}$  and  $\psi_{sz}$ ) in January. Similarly, *C. tribuloides* had the lowest values of  $\psi_{sf}$  and  $\psi_{sz}$  in November. Mean  $\psi_{sz}$  and  $\psi_{sf}$  ranged from -3.75 MPa to -5.09 MPa and from -2.34 MPa to -3.2 MPa respectively in *C. tribuloides*. These values were lower than those reported by Adhikari (2000). *Q. leucotrichophora* showed osmotic adjustment ranging from -2.41 to -3.55 MPa at full turgor and -3.75 to -5.0 MPa at zero turgor. In North American oaks, osmotic adjustment was -0.36 to -3.25 MPa at full turgor and -0.99 to -3.10 MPa at zero turgor (Abrams, 1990). Similarly, Singh *et al.*, (2000) reported a lower range of osmotic adjustment up to -3.5 MPa at  $\psi_{sf}$  and -4.2 MPa at  $\psi_{sz}$  during the dry months of 1999 in Central Himalayan oaks namely *Q. leucotrichophora* and *Q. floribunda*. Osmotic adjustment during dry periods has also been demonstrated in many species of *Quercus* in Europe (*Q. robur* by Osonubi and Davis, 1978), in Nepal (*Q. lanata* and *Q. semecarpifolia* by Poudyal *et al.*, 2012a).

$RWC_z$ , which is a measure of the plant's ability to maintain turgor in the presence of increasing water deficits, did not correlate with soil moisture content in the studied species thus suggesting a possible increase in water uptake from soil through deep roots as suggested by Poudyal *et al.*, (2012a).  $RWC_z$  was higher during September and lower during March in both species. Plant maintains turgidity during dry period by lowering relative water content at zero turgor ( $RWC_z$ ). In *C. tribuloides*,  $RWC_z$  ranged from 82.99 % (March) to 90.15 % (September) whereas in *Q. leucotrichophora*, it ranged from 87.11 % (March) to 91.36 % (September).  $RWC_z$  was lower and tissue elasticity was higher in the same period in *C. tribuloides* due to increased elasticity. This facilitated the plant to continue growth (as leaf flushing) in such dry months. Similar result was shown by Poudyal *et al.*, (2012a). Comparatively *C. tribuloides* had higher elasticity of cell walls than *Q. leucotrichophora* which lost turgor at 87.11 % relative water content while *C. tribuloides* at 82.99 %. The capacity to maintain a high  $RWC_z$  under drought was observed by Zlatev (2005) and Gorai *et al.*, (2010). In *C. tribuloides*,  $RWC_z$  showed slightly negative correlation with  $\psi_{sz}$  and  $\psi_{sf}$  which suggests that osmotic adjustment might not contribute in turgor maintenance while in *Q. leucotrichophora*,  $RWC_z$  showed a strong negative correlation with  $\psi_{sz}$  and  $\psi_{sf}$  ( $r = -0.84$  and  $r = -0.91$ ) respectively.

A significant correlation between  $RWC_z$  and  $\epsilon$  ( $r=0.74$ ) in *Q. leucotrichophora* and ( $r=0.54$ ) in *C. tribuloides* suggested greater elastic adjustment instead of osmotic adjustment. Elasticity of cell wall is the next important pre-requisite for phenological processes occurring mostly in drought seasons. The bulk modulus of elasticity ( $\epsilon$ ) which is inversely proportional to the index of elasticity of cell wall was low in *C. tribuloides* and high in *Q. leucotrichophora* in the dry months. Lower  $\epsilon$  value during drought indicates tissue ability to shrink more during dehydration and elastic adjustment results from the modification of cell walls, which make them more elastic thereby enabling tissue shrinkage during dehydration.

The index of elasticity ( $\epsilon$ ) was least when leaf emergence was progressing and highest when the leaves matured in *C. tribuloides*. Thereafter with the beginning of senescence  $\epsilon$  began to decline which was similar to the result reported by Adhikari (2000). However,  $\epsilon$  reached to its maximum value in August and attained peak during September. The role of  $\epsilon$  in turgor maintenance is considered to be controversial (Cosgrove, 1988). With increasing drought stress,  $\epsilon$  has been found to remain constant (Hsaio *et al.*, 1976), to decrease (Robichaux, 1984) and to increase (Mulkey *et al.*, 1991).

Index of desiccation tolerance (IDT) in both species was highest during dry periods when leaf production and twig growth took place and lowest in monsoon and post monsoon period. Desiccation tolerance is associated with low  $\psi_{sz}$ , low  $RWC_z$  and low  $\epsilon$  (high elasticity) (Lambers *et al.*, 1998). Poudyal *et al.*, (2012a) has also reported high IDT in some Himalayan tree species in the dry months.

Hydraulic conductance of the plants was studied through the parameters like Huber value ( $H_v$ ), Xylem conductivity ( $K_h$ ), Specific conductivity ( $K_s$ ) and leaf specific conductivity ( $K_l$ ). These parameters showed monthly variation in both species.

Huber value ( $H_v$ ) which is considered as relative conducting area is high in plants that lose large quantities of water in transpiration (Larcher, 1995). Both species maintained increased Huber value ( $H_v$ ) in dry months. It might be due to rapid supply of water to newly formed leaf. Leaf senescence might be responsible for high  $H_v$  value in February-March in *Q. leucotrichophora*. Both the studied species showed lower  $H_v$  during September as it showed negative correlation with moisture content,

RWC<sub>z</sub> and  $\epsilon$  and positive correlation with IDT in *C. tribuloides* while negatively correlated with only moisture content in *Q. leucotrichophora*.

Xylem conductivity ( $K_h$ ) in both species was highest in August and lowest in April. In *C. tribuloides*, the lower values of  $K_h$  in dry summer might be because of lesser availability of water in the soil. High  $K_h$  during monsoon might be because of wet soil as it showed positive correlation with moisture content and RWC<sub>z</sub>. In *Q. leucotrichophora*, the value of  $K_h$  was slightly high in February indicating its ability to supply ample amount of water to newly emerged leaves even though the soil moisture content was low. Similar results were obtained by Adhikari (2000) in *C. tribuloides*.

The specific conductivity ( $K_s$ ) is the porosity of wood (Tyree and Ewers, 1996) and depends on the diameter of conducting element (Larcher, 1995).  $K_s$  declined in both species with the beginning of dry months as observed by Prior and Eamus (2000) and Poudyal *et al.*, (2003). Both species showed high  $K_s$  value in monsoon period. It might be because of the development of summer wood and increment in the vessel diameter.  $K_s$  in both species showed positive correlation with soil moisture content.

Leaf specific conductivity ( $K_l$ ), which is a practical measurement of the xylem's hydraulic supply capacity (Zimmerman, 1978) decreased with decreasing water availability.  $K_l$  was recorded lowest in April in both species. The reduction of  $K_l$  due to dehydration can be due to reduction in the permeability of the extra-xylary tissues (Sack and Holbrook 2006, Pou *et al.*, 2013) or collapse of xylem cells (Brodribb and Holbrook 2005).  $K_l$  was high during monsoon. It might be to cope with developing leaves and flowers as well as efficient water in monsoon (Adhikari, 2000).  $K_l$  values ranged from  $0.36 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  to  $1.3 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  in *C. tribuloides* while in *Q. leucotrichophora*, the values ranged from  $0.39 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$  to  $1.22 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ . These values were higher than those of temperate trees observed by Tyree *et al.*, (1991) *Thuja occidentalis* ( $0.05 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) and the angiosperm *Acer sachharum* ( $1.0 \times 10^{-4} \text{ kg m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) but were much lower than the values reported by Poudyal (2004) in *Q. lanata* and *Q. semecarpifolia*.

Starch percentage content in twigs, is the parameter which showed an apparent relationship with leaf phenology. In both species high starch accumulation was found



in zero-year twig at the time of leaf emergence. Such high value was due to the accumulation of reserved carbohydrates as suggested by Poudyal *et al.*, (2012b) in Himalayan tree species. As growth starts, so formed starch was used up by plants. Mostly a higher starch staining was found in current year's twigs than in the previous year's twigs in these species which indicated quick and efficient shift of such accumulates to the new shoots. Similar result was found by Poudyal (2014) in *Castanopsis indica* in Phulchowki hill which stated the indication of expeditious shift of starch accumulates to the new shoots. A correlation in the starch content of 1- year-old and 2- year- old twigs of Pine and *Q. leucotrichophora* was observed by Zobel (1996).

Decrease in starch staining after leaf emergence also indicated hydrolysis of photosynthates into soluble carbohydrates. However a substantial amount of starch was present in the zero- year twig in *C. tribuloides* except in June. According to Larcher (1995) about a third of the reserve material is utilized in the unfolding of leaves and flowers and developing of fruits. As flowering and fruiting took place immediately after leaf flush in the studied species, existing starch might be used for flowering and fruiting by them. *Q. leucotrichophora* with its multiple leaf flushing behavior showed an inconsistent starch staining where the reserve starch was completely hydrolyzed throughout the monsoon and winter period except in August in zero year twigs. This suggested that the reserve starch compensated all the phenological events indicating continuous depletion which might contribute to osmotic regulation during these months (Poudyal, 2004). Epron and Dreyer (1996) suggested that large increase in soluble carbohydrates contributes in the osmotic adjustment of oaks (*Q. petraea* Matt. Liebl.).

In both the studied species, SLM was highest during February and lowest values were recorded during the period of leaf fall i.e. April. Variations in SLM are caused due to variations in leaf thickness or in leaf mass density (Witkowski and Lamont 1991) and are related with the differences in both leaf anatomy and chemical composition (Lambers *et al.*, 1998). The differences in SLM have been related to the differences in the proportion of photosynthetic tissues. Poudyal *et al.*, (2012b) suggested an increase in SLM as leaves expanded which was due to the increased thickness of palisade parenchyma. The value of SLM was found to be increased during monsoon and post monsoon period when leaves were fully developed and decreased when leaf

emergence and simultaneously senescence took place during April. Senescence occurred by drying up, damages and holes in leaves which lead to declined leaf density thereby lowering SLM as stated by Poudyal *et al.*, (2012b). A positive correlation between SLM and Huber value in *Q. leucotrichophora* indicated that changes in SLM was related to the changes in leaf area (Poudyal *et al.*, 2012b).

Abiotic factors such as periodicity of rainfall and soil water availability or variations in photoperiod usually have direct impacts on plant phenology. Increased water stress during early dry season generally causes a decline in water potential and induces leaf shedding (Borchert, 1995). Phenological events in *Q. leucotrichophora* commenced mostly from early March and ended before monsoon while in *C. tribuloides* flowering started from September. In *C. tribuloides*, different workers have reported flowering and fruiting in different months. Subedi (1999) has reported the flowering period during May to June and fruiting during June to August. Flora of Kathmandu valley has reported August to September as the flowering period and August to October fruiting period. Adhikari (2000) has reported flowering from May to June and June to September as the period of fruiting. *C. tribuloides* produced additional leaves in early winter month (November) while *Q. leucotrichophora* showed multiple leaves flushing, both species having damaged leaves all through. Similar result was reported by Poudyal *et al.*, (2012b) in *C. indica* and *Q. lanata* which produced additional leaves by using photosynthates from the first formed leaves and in this way the species might compensate for the large proportion of leaf damaged throughout the year.

In *Q. leucotrichophora*, the phenological events such as leaf drop, leaf emergence flowering and fruiting occurred in the dry summer months as in the temperate trees of Indian Central Himalaya (Ralhan *et al.*, 1985). Both species showed evergreen types of leaf exchange pattern with a concentrated leaf drop in a particular season but plants were not naked due to simultaneous leafing. In studied species, concentrated leaf fall during March- April reduced transpirational water loss and could help to rehydrate the tissue (Shrestha *et al.*, 2006). The new leaf production and expansion started from March onwards. An incessant leaf shedding shown by both species with little change in SLM in during the dry period could be a way to recover its water status mainly by decreasing exposed leaf area rather than by leaf morphological changes (Poudyal *et al.*, 2012b).

Leaf abscission is a functional strategy of the plants to maintain a favourable tissue water status because decreased leaf surface area eliminates higher fraction of transpirational water loss as reported by Poudyal *et al.*, (2012b). SLM decreased in both species at the time of senescence. Senescence occurred by yellowing, drying up, damages and holes and such damages and drying of the leaves prior to senescence decreased the leaf density thus lowering SLM. The positive correlation between SLM and Huber value in *Q. leucotrichophora* indicated that changes in SLM was related to the changes in leaf area (Poudyal *et al.*, 2012b).

The leaf area which determines light interception is an important variable in determining plant productivity (Gifford *et al.*, 1984, Koester *et al.*, 2014). In *Q. leucotrichophora*, the maximum leaf area was found during September when the leaf development was at its peak while the lowest value was found during February which might be associated with newly developing leaves and damaged old leaves. Fully expanded leaf had higher leaf area thus indicating higher rate of photosynthesis (Kikuzawa, 1995) found in this species. Significant decrease in leaf area was also indicated by highest value of  $H_v$  during dry summer month. A rapid increase in leaf area with lower SLM after rainy season was reported by Poudyal *et al.*, (2012b) in some Himalayan species. They attributed the increased of parenchymatous tissues rather than sclerophylly of the leaves as adequate amount of water was present in the soil at that time. However, in *C. tribuloides* the average leaf area was highest during March and lowest during June which could be associated with their timing of maturation. The maximum photosynthesis during March might be low since the rate of photosynthesis declined with ageing of leaves as stated by Kikuzawa (1995). Lowest mean leaf area in June might be due to the new leaves were still in maturing stage. Exponential increase in leaf area would have been expected for plants growing in optimum conditions (Goudriaan and Van Laar, 1994).

In the study site, *C. tribuloides* was found growing in moisture- rich and shade environment whereas *Q. leucotrichophora* inhabited sunlight exposed slope. Comparatively, *C. tribuloides* had larger leaf area than *Q. leucotrichophora* throughout the observation period. Shade leaves had a larger leaf area but low SLM indicating small sclerenchyma and less vascular bundle. A lower light saturation point of the overall photosynthesis compared to leaves exposed to full sunlight was reported by Bjorkman 1981, Cao and Booth 2001). Furthermore, the variation in the leaf

morphology (shape and size) in studied species might better reflect the changes in environmental factors such as temperature, light intensity, and water status.

## CHAPTER 6: CONCLUSION

Both *Castanopsis tribuloides* and *Quercus leucotrichophora* showed that the climatic circumstances and monthly variation had strong control over water status of plants. In both species, marked variation in water relation parameters were observed along with different months. The phenological activities in the studied species occurred during the summer drought although fruiting is prolonged up to winter. Both species were moderately stressed during phenologically active period (dry periods of the year) in the sub-tropical forest ecosystems. Phenomorphology of these species showed a close affinity to their internal water status and turgor maintenance.

February was found to be the most drought affected month during the study, which was supported by low soil moisture percentage during that month among sampled species. Water stress may be a precursor including phenological activities in the plant. It showed internal adjustment as well as leaf abscission to buffer water stress to the growing tissues during drought. It lowered transpirational water loss for the proper retention of water. Increased water stress during early dry season generally causes a decline in water potential and induces leaf shedding. A leaf abscission is morphological response to substantial drought. During drought, it assessed water from deeper soil.

Both the phenological processes (leaf fall and emergence) and drought occurred at the same time among sampled species. So, during that time low index of elasticity ( $\epsilon$ ) and low value of relative water content at zero turgor ( $RWC_z$ ) were found during the cell growth and development. In both studied species, elastic adjustment rather than osmotic adjustment was found considered to be functional and most effective strategy of drought tolerance. These plant species also maintain turgor in response to water stress by the drought avoidance mechanism such as leaf shedding and partial defoliation. Thus, the studied species showed two extreme responses, identified from the continuum of adaptive strategies to survive the drought. The first is a drought avoidance strategy as evident from shedding of their foliage in dry summer and second is drought resistance strategy as growth continues even during dry season as shown by both sampled species in my study site.

Moreover, the lower value of  $\varepsilon$  in the drought period made the flowering and fruiting smooth. Lower value of  $RWC_z$  was also important in maintaining turgor for plant growth during dry months. The monthly variation in xylem conductance occurred in studied species. Xylem conductivity showed lower value during dry months when leaf abscission was in progressed.

Reserved starch percentage and SLM had a strong effect on phenological activities. They increased during the vegetative bud break and leaf emergence and decreased during senescence. Starch was found to accumulate for the biosynthetic processes during the period of phenological activities. Leaf emergence period was found to be completed with the complete exhaustion of reserved starch among studied species. SLM was highest for matured leaves and lowest for senescensing leaves.

Tree species may postpone or tolerate dehydration by elastic and osmotic adjustment. Thus, it is concluded that the studied species showed both drought tolerance and avoidance mechanism thus enabling them to withstand the typical seasonality of precipitation and unprecedented drought of this region.

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## APPENDICES

**Appendix 1:** Soil moisture content (%) at 15 cm and 30 cm depth, monthly variation for *C. tribuloides* and *Q. leucotrichophora*. Values are mean with  $\pm$  Standard error.

Species	Months	SMC <sub>15cm</sub> (%)	SMC <sub>30cm</sub> (%)
<i>C. tribuloides</i>	<b>Feb.</b>	10.10 $\pm$ 1.20	10.53 $\pm$ 1.18
	<b>Mar.</b>	15.35 $\pm$ 2.50	17.24 $\pm$ 3.61
	<b>Apr.</b>	13.40 $\pm$ 1.30	15.32 $\pm$ 3.31
	<b>Jun.</b>	20.40 $\pm$ 0.79	20.14 $\pm$ 1.42
	<b>Aug.</b>	19.92 $\pm$ 0.90	20.51 $\pm$ 1.43
	<b>Sep.</b>	23.79 $\pm$ 0.46	23.05 $\pm$ 1.32
	<b>Nov.</b>	12.13 $\pm$ 0.90	11.90 $\pm$ 1.16
	<b>Jan.</b>	12.50 $\pm$ 1.06	15.11 $\pm$ 0.60
<i>Q. leucotrichophora</i>	<b>Feb.</b>	5.53 $\pm$ 0.37	6.70 $\pm$ 0.47
	<b>Mar.</b>	12.44 $\pm$ 2.16	15.44 $\pm$ 3.91
	<b>Apr.</b>	14.14 $\pm$ 2.73	14.70 $\pm$ 2.84
	<b>Jun.</b>	17.06 $\pm$ 3.05	16.13 $\pm$ 3.20
	<b>Aug.</b>	15.56 $\pm$ 0.20	13.21 $\pm$ 1.71
	<b>Sep.</b>	15.31 $\pm$ 0.12	14.73 $\pm$ 0.97
	<b>Nov.</b>	6.60 $\pm$ 1.25	6.59 $\pm$ 1.14
	<b>Jan.</b>	12.58 $\pm$ 1.83	11.20 $\pm$ 2.15



**Appendix 2:** Parameters obtained PV- curve analysis. Monthly variation in relative water content at zero turgor ( $RWC_z$ , %), osmotic potential at zero turgor ( $\psi_{sz}$ , MPa), osmotic potential at full turgor ( $\psi_{sf}$ , MPa), index of elasticity ( $\epsilon$ , MPa) and desiccation tolerance (IDT) for *C. tribuloides* and *Q. leucotrichophora*. Values are mean with  $\pm$  Standard error.

Species	Months	$RWC_z$	$\psi_{sz}$	$\psi_{sf}$	$\epsilon$	IDT
<i>C. tribuloides</i>	Feb.	88.0 $\pm$ 2.76	-4.0	-2.34 $\pm$ 0.03	2.15 $\pm$ 0.50	62.28
	Mar.	82.99 $\pm$ 0.34	-4.90 $\pm$ 0.05	-3.14 $\pm$ 0.16	2.14 $\pm$ 0.33	77.29
	Apr.	84.93 $\pm$ 0.63	-4.16 $\pm$ 0.08	-2.52 $\pm$ 0.13	1.69 $\pm$ 0.15	74.53
	Jun.	88.20 $\pm$ 1.11	-4.04 $\pm$ 0.31	-2.56 $\pm$ 0.13	2.48 $\pm$ 0.11	60.96
	Aug.	88.54 $\pm$ 1.12	-3.75 $\pm$ 0.04	-2.43 $\pm$ 0.19	2.52 $\pm$ 0.60	39.83
	Sep.	90.15 $\pm$ 0.43	-4.15 $\pm$ 0.19	-2.81 $\pm$ 0.21	3.15 $\pm$ 0.14	40.45
	Nov.	89.30 $\pm$ 1.12	-5.09 $\pm$ 0.08	-3.20 $\pm$ 0.02	3.13 $\pm$ 0.55	26.42
	Jan.	88.53 $\pm$ 0.43	-4.37 $\pm$ 0.09	-3.02 $\pm$ 0.19	3.08 $\pm$ 0.02	42.30
<i>Q. leucotrichophora</i>	Feb.	89.98 $\pm$ 0.89	-4.86 $\pm$ 0.21	-3.23 $\pm$ 0.15	3.43 $\pm$ 0.50	27.41
	Mar.	87.11 $\pm$ 0.90	-4.44 $\pm$ 0.13	-3.21 $\pm$ 0.19	3.11 $\pm$ 0.69	70.02
	Apr.	87.66 $\pm$ 1.92	-4.47 $\pm$ 0.26	-2.75 $\pm$ 0.19	2.66 $\pm$ 0.55	55.79
	Jun.	88.0 $\pm$ 0.22	-4.45 $\pm$ 0.05	-2.92 $\pm$ 0.08	2.88 $\pm$ 0.43	51.89
	Aug.	90.02 $\pm$ 0.98	-3.75 $\pm$ 0.04	-2.41 $\pm$ 0.19	2.62 $\pm$ 0.36	40.38
	Sep.	90.86 $\pm$ 0.41	-4.75 $\pm$ 0.24	-3.34 $\pm$ 0.12	3.77 $\pm$ 0.05	21.57
	Nov.	90.13 $\pm$ 0.80	-4.90 $\pm$ 0.05	-2.89 $\pm$ 0.21	2.87 $\pm$ 0.34	33.16
	Jan.	90.97 $\pm$ 0.50	-5.0	-3.55 $\pm$ 0.08	3.11 $\pm$ 0.69	22.63

**Appendix 3:** Parameters obtained from twig conductance. Monthly variation in Xylem conductivity ( $K_h$ , kg. m. sec<sup>-1</sup> . MPa<sup>-1</sup> ), Huber Value ( $H_v$ ), Specific conductivity ( $K_s$ , kg. m<sup>-1</sup>. sec<sup>-1</sup>. MPa<sup>-1</sup>) and Leaf specific conductivity ( $K_l$ , kg. m<sup>-1</sup>. sec<sup>-1</sup>.MPa<sup>-1</sup>) for *C. tribuloides* and *Q. leucotrichophora*. Values are mean with  $\pm$  Standard error.

Species	Months	$H_v$	$K_h$	$K_s$	$K_l$
<i>C. tribuloides</i>	<b>Feb.</b>	1.92*10 <sup>-4</sup> ±0.32*10 <sup>-4</sup>	1.04*10 <sup>-6</sup> ±0.16*10 <sup>-6</sup>	0.46±0.15	0.75*10 <sup>-4</sup> ±0.25*10 <sup>-4</sup>
	<b>Mar.</b>	2.06*10 <sup>-4</sup> ±0.46*10 <sup>-4</sup>	1.19*10 <sup>-6</sup> ±0.18*10 <sup>-6</sup>	0.42±0.02	0.67*10 <sup>-4</sup> ±0.13*10 <sup>-4</sup>
	<b>Apr.</b>	1.90*10 <sup>-4</sup> ±0.37*10 <sup>-4</sup>	0.64*10 <sup>-6</sup> ±0.03*10 <sup>-6</sup>	0.31±0.03	0.36*10 <sup>-4</sup> ±0.04*10 <sup>-4</sup>
	<b>Jun.</b>	1.57*10 <sup>-4</sup> ±0.06*10 <sup>-4</sup>	1.16*10 <sup>-6</sup> ±0.20*10 <sup>-6</sup>	0.34±0.11	0.43*10 <sup>-4</sup> ±0.11*10 <sup>-4</sup>
	<b>Aug.</b>	1.72*10 <sup>-4</sup> ±0.41*10 <sup>-4</sup>	2.88*10 <sup>-6</sup> ±1.49*10 <sup>-6</sup>	1.08±0.68	1.38*10 <sup>-4</sup> ±0.79*10 <sup>-4</sup>
	<b>Sep.</b>	1.42*10 <sup>-4</sup> ±0.38*10 <sup>-4</sup>	1.44*10 <sup>-6</sup> ±0.34*10 <sup>-6</sup>	0.80±0.10	0.74*10 <sup>-4</sup> ±0.15*10 <sup>-4</sup>
	<b>Nov.</b>	1.42*10 <sup>-4</sup> ±0.38*10 <sup>-4</sup>	1.17*10 <sup>-6</sup> ±0.05*10 <sup>-6</sup>	0.33±0.05	0.97*10 <sup>-4</sup> ±0.15*10 <sup>-4</sup>
	<b>Jan.</b>	1.89*10 <sup>-4</sup> ±0.32*10 <sup>-4</sup>	1.37*10 <sup>-6</sup> ±0.15*10 <sup>-6</sup>	0.78±0.03	1.16*10 <sup>-4</sup> ±0.14*10 <sup>-4</sup>
<i>Q. leucotrichophora</i>	<b>Feb.</b>	3.12*10 <sup>-4</sup> ±0.89*10 <sup>-4</sup>	2.25*10 <sup>-6</sup> ±0.33*10 <sup>-6</sup>	0.45±0.04	1.17*10 <sup>-4</sup> ±0.39*10 <sup>-4</sup>
	<b>Mar.</b>	2.37*10 <sup>-4</sup> ±0.73*10 <sup>-4</sup>	1.77*10 <sup>-6</sup> ±0.84*10 <sup>-6</sup>	0.56±0.21	1.05*10 <sup>-4</sup> ±0.60*10 <sup>-4</sup>
	<b>Apr.</b>	1.68*10 <sup>-4</sup> ±0.26*10 <sup>-4</sup>	1.02*10 <sup>-6</sup> ±0.25*10 <sup>-6</sup>	0.38±0.16	0.39*10 <sup>-4</sup> ±0.12*10 <sup>-4</sup>
	<b>Jun.</b>	1.36*10 <sup>-4</sup> ±0.11*10 <sup>-4</sup>	2.63*10 <sup>-6</sup> ±0.53*10 <sup>-6</sup>	0.78±0.22	0.89*10 <sup>-4</sup> ±0.20*10 <sup>-4</sup>
	<b>Aug.</b>	2.34*10 <sup>-4</sup> ±0.60*10 <sup>-4</sup>	3.07*10 <sup>-6</sup> ±1.05*10 <sup>-6</sup>	0.60±0.05	1.22*10 <sup>-4</sup> ±0.31*10 <sup>-4</sup>
	<b>Sep.</b>	1.06*10 <sup>-4</sup> ±0.27*10 <sup>-4</sup>	1.38*10 <sup>-6</sup> ±0.59*10 <sup>-6</sup>	0.61±0.23	0.44*10 <sup>-4</sup> ±0.08*10 <sup>-4</sup>
	<b>Nov.</b>	2.02*10 <sup>-4</sup> ±0.41*10 <sup>-4</sup>	1.45*10 <sup>-6</sup> ±0.37*10 <sup>-6</sup>	0.49±0.21	0.68*10 <sup>-4</sup> ±0.20*10 <sup>-4</sup>
	<b>Jan.</b>	1.36*10 <sup>-4</sup> ±0.14*10 <sup>-4</sup>	1.31*10 <sup>-6</sup> ±0.22*10 <sup>-6</sup>	0.53±0.10	0.56*10 <sup>-4</sup> ±0.06*10 <sup>-4</sup>

**Appendix 4:** Monthly variation in mean leaf area (mm<sup>2</sup>) for *C. tribuloides* and *Q. leucotrichophora*.

Species	Months	Mean leaf area (mm <sup>2</sup> )
<i>C. tribuloides</i>	Feb.	410.07
	Mar.	468.33
	Apr.	340.70
	Jun.	336.73
	Aug.	381.27
	Sep.	385.24
	Nov.	402.30
	Jan 2	382.76
	Jan 28	375.25
<i>Q. leucotrichophora</i>	Feb.	268.91
	Mar.	281.87
	Apr.	315.17
	Jun.	335.18
	Aug.	344.39
	Sep.	348.64
	Nov.	340.99
	Jan 2	339.64
	Jan 28	335.43

**Appendix 5:** Monthly variation in starch reserved (%) and specific leaf mass (SLM,  $\text{gcm}^{-2}$ ) for *C. tribuloides* and *Q. leucotrichophora*. Values are mean with  $\pm$  Standard error.

Species	Months	Starch reserved		SLM
		0 Year	1 Year	
<i>C. tribuloides</i>	Feb.	30	0 $\pm$ 0	1.13*10 <sup>-2</sup> $\pm$ 0.05*10 <sup>-2</sup>
	Mar.	100	61.67 $\pm$ 1.67	1.0*10 <sup>-2</sup> $\pm$ 0.12*10 <sup>-2</sup>
	Apr.	47.67 $\pm$ 1.45	29.33 $\pm$ 0.67	0.62*10 <sup>-2</sup> $\pm$ 0.08*10 <sup>-2</sup>
	Jun.	0 $\pm$ 0	0 $\pm$ 0	0.86*10 <sup>-2</sup> $\pm$ 0.06*10 <sup>-2</sup>
	Aug.	11.67 $\pm$ 1.67	0 $\pm$ 0	0.99*10 <sup>-2</sup> $\pm$ 0.07*10 <sup>-2</sup>
	Sep.	9.33 $\pm$ 0.67	0 $\pm$ 0	0.90*10 <sup>-2</sup> $\pm$ 0.07*10 <sup>-2</sup>
	Nov.	90	80	1.01*10 <sup>-2</sup> $\pm$ 0.04*10 <sup>-2</sup>
	Jan	27.67 $\pm$ 1.45	0 $\pm$ 0	0.91*10 <sup>-2</sup> $\pm$ 0.06*10 <sup>-2</sup>
<i>Q. leucotrichophora</i>	Feb.	31 $\pm$ 2.08	20	1.31*10 <sup>-2</sup> $\pm$ 0.07*10 <sup>-2</sup>
	Mar.	100	80	0.88*10 <sup>-2</sup> $\pm$ 0.10*10 <sup>-2</sup>
	Apr.	30	10	0.80*10 <sup>-2</sup> $\pm$ 0.06*10 <sup>-2</sup>
	Jun.	0 $\pm$ 0	0 $\pm$ 0	1.00*10 <sup>-2</sup> $\pm$ 0.11*10 <sup>-2</sup>
	Aug.	30	0 $\pm$ 0	1.10*10 <sup>-2</sup> $\pm$ 0.05*10 <sup>-2</sup>
	Sep.	0 $\pm$ 0	0 $\pm$ 0	1.09*10 <sup>-2</sup> $\pm$ 0.05*10 <sup>-2</sup>
	Nov.	0 $\pm$ 0	0 $\pm$ 0	1.10*10 <sup>-2</sup> $\pm$ 0.04*10 <sup>-2</sup>
	Jan	0 $\pm$ 0	0 $\pm$ 0	1.06*10 <sup>-2</sup> $\pm$ 0.07*10 <sup>-2</sup>

## PHOTOPLATES





Young new leaves in *C. tribuloides* in September



Inflorescence emergence in *C. tribuloides* in September





Leaves damaged by insects and necrotic patches in *C. tribuloides*



A twig of *Q. leucotrichophora*



Young fruits of *Q. leucotrichophora* in June