Assessment of the Carbon dioxide sequestration potential of selected tree species using controlled growth chambers

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CERTIFICATE

This is to certify that the Ph.D. thesis titled **"Assessment of the Carbon dioxide sequestration potential of selected tree species using controlled growth chambers",** submitted to the University of Calicut in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Botany by Mrs. Aparna Sreekumar is a bonafide record of research work carried out by her under my guidance and supervision in the Division of Environmental Science, Department of Botany, University of Calicut.

No part of the present work has previously formed the basis for the award of any other Degree, Diploma, Fellowship, or similar title, to any candidate in any University. The modifications suggested by the Research Advisory Committee (Botany) of the University of Calicut have been incorporated into the thesis. It is also affirmed that all corrections and modifications suggested by the adjudicators have been fully incorporated into the thesis.

Calicut University Campus
Dated:

Dr. C. C. Harilal
(Research Supervisor and Guide)

DECLARATION

I hereby declare that the work presented in the thesis entitled "**Assessment of the Carbon dioxide sequestration potential of selected tree species using controlled growth chambers"** is based on the original work done by me under the guidance and supervision of Prof. (Dr.) C.C. Harilal, Head, Department of Botany (Division of Environmental Science), University of Calicut, and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit.

I also declare that the thesis is free from AI-generated content.

It is also affirmed that all corrections and modifications suggested by the adjudicators have been fully incorporated into the thesis.

Signature

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Aparna Sreekumar

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ABBREVIATIONS

%	-	Percentage
µ mol	-	Micromol
CC	-	Control chamber
CES	-	Controlled environment systems
CGT	-	CO_2 gradient tunnel
CH_3COONH_4	-	Ammonium acetate
CHNS	-	Carbon hydrogen nitrogen sulfur
CO_2	-	Carbon dioxide
COP	-	Conference of the Parties
CPP	-	Closed plant production
CTC	-	Closed top chambers
CVOTC	-	Controlled ventilation open-top chamber
df	-	Degree of freedom
dm3/min	-	Decimeter cube per minute
DMSO	-	Dimethyl sulphoxide
DOT	-	Day of Treatment
E	-	Transpiration
EDTA	-	Ethylene diamine tetra acetic acid
ET	-	Elevated ambient temperature
ET	-	Elongated tunnel
FACE	-	Free air Carbon dioxide enrichment systems
FTIR	-	Fourier Transform Infrared Spectroscopy
GHGs	-	Greenhouse gases
gs	-	Stomatal conductance
H_2SO_4	-	Sulphuric acid
HCFCs	-	Hydrochlorofluorocarbons
HCl	-	Hydro chloric acid
HFCs	-	Hydrofluorocarbons
IPCC	-	International Panel on Climate Change
IRGA	-	Infra-red gas analyzer
$K_2Cr_2O_7$	-	Potassium dichromate
KFRI	-	Kerala Forest Research Institute
$KMnO_4$	-	Potassium permanganate
Mg $Cha^{-1}yr^{-1}$	-	Megagram carbon per hectare per year
Na_2CO_3	-	Sodium carbonate
NaOH	-	Sodium hydroxide
^oC	-	Degree Celsius
OTC	-	Open top chambers
PCA	-	Principal Component Analysis

pH	-	Potential of hydrogen
pH	-	Potence hydrometer
PLC	-	Programmable Logic Controller
PPFD	-	Photosynthetic Proton Flux Density
ppm	-	Parts per million
ppm	-	Parts per million
PVC	-	Polyvinyl chloride
RCPs	-	Representative Concentration Pathways
SACC	-	Screen-aided Carbon dioxide control
SAPCC	-	State Action Plan on Climate Change
SCADA	-	Superficiary Control and Data Acquisition
TC	-	Treatment chamber
ton/ha	-	Tonns per hectare
WUE	-	Water use efficiency

ABSTRACT

The present study attempted to assess the CO_2 assimilation efficiencies of six tree species (*Terminalia arjuna*, *Swietenia macrophylla*, *Pongamia pinnata*, *Simarouba glauca*, *Mimusops elengi*, and *Syzygium cumini*) under controlled growth conditions. The experiment was carried out in two growth chambers, each with a volume of 6.32 m^3, constructed with PVC frames and covered with transparent polyvinyl chloride sheets. The control chamber (CC) was equipped with the facility for the supply of ambient air, whereas the treatment chamber (TC) with the facility for the supply of CO_2-air mixture in specific doses using an air compressor and a nebulizer. Both chambers were fitted with the facility for the analysis of CO_2 (NDIR type Infrared Gas Analyzer), temperature (^{0}C), and humidity (%) using a Billion Bag digital wireless electronic hygro-thermometer. Both chambers were also equipped with an exhaust facility at the top along with a semi-automated facility for the irrigation of plantlets during experimentation.

For treatment studies, ca. 1½-year-old saplings of *T. arjuna*, *S. macrophylla*, *P. Pinnata*, *S. glauca*, *M. elengi*, and *S. cumini* were employed separately. For each study, one set of saplings was retained in the CC and the other in the TC and were then closed and sealed from the outside. The CC was supplied daily twice (9 am and 6 pm) with ambient air, maintaining a CO_2 concentration of 475±42 ppm, and the TC with a CO_2-air mixture, maintaining a resultant CO_2 concentration of 979.83±30.93 ppm. The magnitude of CO_2 concentration (ppm) along with temperature and humidity within the chambers was monitored twice a day at 9 a.m. and 6 p.m. The experiment was continued for 15 days. The resultant day and night flux in CO_2 was estimated from these results of CO_2. A standardization study was also undertaken in the same way in empty chambers (without plants) and the results were used for the estimation of gross and net flux in CO_2 associated with the respective tree species. During experimentation the growth (plant height, stem diameter, leaf length, leaf breadth, leaf number, and leaf area and biomass) and biochemical changes (pigments such as chlorophyll a, b, total chlorophyll, carotenoids; plant metabolites such as carbohydrates, protein, and phenol; minerals such as calcium, magnesium, sodium and

potassium and carbon and nitrogen) owing to varying levels of CO_2 supply at specific stages of growth were assessed. The results are statistically validated.

The study revealed that the CO_2 assimilation potentials of *Swietenia macrophylla* are higher, followed by *Terminalia arjuna*, *Pongamia pinnata*, *Simarouba glauca*, *Syzygium cumini*, and *Mimusops elengi*. The species *Swietenia macrophylla* is found to be more efficient in carbon sequestration, due to its increased CO_2 assimilation, lower respiratory release, increased biomass content, increased growth characteristics, metabolites, and nutrients. The species can be considered by policymakers and urban planners for its inclusion in various carbon offset planting initiatives. However, the present outcomes are based on a laboratory-based analysis and hence field-level or site-specific validation of the species is required for further confirmation of their Carbon sequestration potentials.
Keywords: Controlled growth chambers, elevated CO_2, diurnal flux, growth and biochemical response, carbon sequestration,

സംഗ്രഹം

വൃക്ഷങ്ങളുടെ കാർബൺഡയോക്സൈഡ് ആഗിരണക്ഷമത നിർണ്ണയിക്കുക എന്നതായിരുന്നു ഈ പഠനത്തിന്റെ ലക്ഷ്യം. ഇതിനായി 6 ഇനം വൃക്ഷതൈകളെയാണ് ഉപയോഗപ്പെടുത്തിയത്. ഒന്നരവർഷം പ്രായമായ നീർമരുത് (*Terminalia arjuna*) മഹാഗണി (*Swietenia Macrophylla*), ഉങ്ങ് (*Pongamia Pinnata*), ലക്ഷ്മിതരു (*Simaraouba glauca*), ഇലഞ്ഞി (*Mimusops elengi*), ഞാവൽ (*syzygium cumini*) എന്നിവയാണ് അവ.

പരീക്ഷിക്കുന്നതിനായി രൂപകൽപ്പന ചെയ്ത 6.32m^3 വ്യാപ്തമുള്ള ചേംബറിന്റെ ചട്ടക്കൂടുകൾ പി.വി.സി പൈപ്പുകൾ ഉപയോഗിച്ച് നിർമ്മിച്ചതും സുതാര്യമായ പി.വി.സി ഷീറ്റുകളാൽ ഭദ്രമായി മൂടി അടച്ചിട്ടുള്ളതുമാണ്. എയർ കംപ്രസ്സറിന്റെയും നെബുലൈസറിന്റെയും സഹായത്തോടെ അന്തരീക്ഷവായു കൺട്രോൾ ചേംബറിലും (സി.സി) കാർബൺ ഡയോക്സൈഡ് അടങ്ങിയ വായുമിശ്രിതം ട്രീറ്റ്മെന്റ് ചേംബറിലും (ടി.സി) നൽകുവാനുള്ള സൗകര്യം പ്രത്യേകമായി സജ്ജീകരിച്ചു. രണ്ട് ചേംബറുകളിലേയും കാർബൺഡയോക്സൈഡ് അളക്കാനായി ഇൻഫ്രാറെഡ് ഗ്യാസ് അനലൈസറും താപനില (^{o}c), ഈർപ്പം (%) എന്നിവ നിർണ്ണയിക്കാനായി ഇലക്ട്രോണിക് ഹൈഗ്രോ തെർമോമീറ്ററും ഉപയോഗപ്പെടുത്തി. ചെടികൾ നനയ്ക്കുന്നതിനായി സെമിഓട്ടോമേറ്റഡ് സൗകര്യവും ചേംബറിന്റെ മുകൾഭാഗത്ത് എക്സ്ഹോസ്റ്റ് സൗകര്യവും സജ്ജീകരിച്ചിട്ടുണ്ട്.

എല്ലാ വൃക്ഷങ്ങളുടെയും ഓരോ കൂട്ടം തൈകൾ വീതം സി.സി.യിലും ടി.സിയിലും വളർത്തി. സി.സിയിൽ ദിവസേന രണ്ടുപ്രാവശ്യം (9am, 6pm) അന്തരീക്ഷ വായു നൽകുകയും കാർബൺ ഡയോക്സൈഡ് സാന്ദ്രത 475$\pm$42ppm എന്ന നിലയിൽ നിലനിർത്തുകയും ചെയ്തു. എന്നാൽ ടി.സിയിൽ അന്തരീക്ഷവായുവിനൊപ്പം കാർബൺഡയോക്സൈഡ് കൂടി നൽകുകയും ആയതിന്റെ സാന്ദ്രത 979.83$\pm$30.93ppm ആയി നിലനിർത്തുകയും ചെയ്തു. കാർബൺ ഡയോക്സൈഡിന്റെ സാന്ദ്രതയും താപനിലയും ആപേക്ഷിക ഈർപ്പവും ദിവസേന രാവിലെ ഒമ്പതിനും വൈകീട്ട് ആറിനും രേഖപ്പെടുത്തി. പകലും രാത്രിയും ഉള്ള CO_2 ഇങ്ങനെയാണ് നിർണ്ണയിച്ചത്. ഇങ്ങിനെ 15 ദിവസം തുടർന്നു.

ഇതോടൊപ്പം തന്നെ ചെടികളില്ലാതെ ഇതേ പരീക്ഷണം സ്റ്റാൻഡെസേഷൻ എന്ന നിലയിൽ വേറിട്ടും നടത്തിയിരുന്നു. കാർബൺഡയോക്സൈഡിന്റെ gross and net flux ഓരോ വൃക്ഷത്തൈകളുടെയും ഈ തരത്തിലാണ് കണ്ടുപിടിച്ചത്. വൃക്ഷത്തൈകളുടെ വളർച്ച (ഉയരം, തണ്ടിന്റെ വണ്ണം, ഇലകളുടെ നീളം, വീതി, എണ്ണം, വിസ്തൃതി, ജൈവാംശം), ജൈവരാസപരമായ മാറ്റങ്ങൾ (പിഗ്മെന്റുകളായ ക്ലോറോഫിൽ, എ, ബി ആകെയുള്ള ക്ലോറോഫിൽ, കരോട്ടിനോയിഡുകൾ) സസ്യ മെറ്റാബൊളൈറ്റുകൾ (കാർബോഹൈഡ്രേറ്റ്, പ്രോട്ടീൻ, ഫിനോൾ) ധാതുക്കൾ (കാൽസ്യം, മഗ്നീഷ്യം, സോഡിയം, പൊട്ടാസ്യം, കോപ്പർ, സിങ്ക്) മൂലകങ്ങൾ (കാർബൺ, നൈട്രജൻ) എന്നിവയിലെ മാറ്റങ്ങൾ വിവിധ വളർച്ചാ ഘട്ടത്തിലും രേഖപ്പെടുത്തി. ഈ ഫലങ്ങൾ സ്റ്റാറ്റിസ്റ്റിക്കൽ രീതികൾ അവലംബിച്ച് സാധൂകരിക്കുകയും ചെയ്തു.

പഠന വിധേയമാക്കിയ വൃക്ഷങ്ങളിൽ മഹാഗണിക്കാണ് കാർബൺഡയോക്സൈഡ് ആഗിരണശേഷി ഏറ്റവും ഉയർന്നത് എന്ന് വ്യക്തമായി. പിന്നാലെ മരുത്, ഉങ്ങ്, ലക്ഷിമിതരു, ഞാവൽ, ഇലഞ്ഞി എന്നീ വൃക്ഷങ്ങളും സ്ഥാനം പിടിച്ചു. മഹാഗണിയുടെ വർദ്ധിച്ച കാർബൺഡയോക്സൈഡ് സ്വാംശീകരണം, ശ്വസനത്തിൽ പുറത്തേക്ക് വിടുന്നതിലുള്ള കുറവ്, കൂടിയ ജൈവാംശം, കൂടിയ വളർച്ച ഘടകങ്ങൾ, മെറ്റാബോളൈറ്റുകൾ, ധാതുക്കൾ എന്നിവയാണ് ഈ വൃക്ഷത്തെ താരതമ്യേന വർദ്ധിച്ച കാർബൺഡയോക്സൈഡ് സ്വാംശീകരണശേഷി ഉള്ളതാക്കുന്നത്. നയരൂപകർത്താക്കൾക്കും നഗര ആസൂത്രകർക്കും ഈ വൃക്ഷത്തെ കാർബൺ ഓഫ്സെറ്റ് നടീൽ വസ്തുവായി കരുതാവുന്നതാണ്.

നിലവിലെ ഫലങ്ങൾ ലബോറട്ടറി അടിസ്ഥാന പഠനത്തിന്റേതാണ്. ആയതിനാൽ സ്ഥിരീകരണത്തിനായി ഫീൽഡ് ലെവൽ പഠനങ്ങൾ ആവശ്യമാണ്.

സൂചകപദങ്ങൾ:

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GENERAL INTRODUCTION

Global warming and climate change, attributed to greenhouse gases (GHGs), are the most serious environmental concerns of the present millennium. The main greenhouse gases include Carbon dioxide, Methane, Nitrous oxide, Hydrochlorofluorocarbons (HCFCs), Hydrofluorocarbons (HFCs), and Ozone in the lower atmosphere. Human activities have been the main contributor of greenhouse gases primarily due to deforestation, land use changes, and the burning of fossil fuels. Apart from anthropogenic sources, greenhouse gases are contributed by natural sources such as volcanoes, forest fires, and hot springs (Tuckett, 2021).

CO_2 is the primary greenhouse gas emitted through human activities and it accounts for 70-80% of the total emissions (IPCC, 2007). CO_2 is considered an important attribute of climate change as it remains in the atmosphere for a long period, and traps heat within the atmosphere by absorbing the Sun's energy and preventing it from escaping into space (Archer *et al*., 2009). Plants and other microscopic organisms uptake CO_2 and are utilized for the synthesis of carbohydrates and other molecules, which are essential for growth and other metabolic activities. The carbon cycle is completed when these carbohydrates are converted back into CO_2 and water by decomposition (Archer, 2011). Both natural and human activities cause emissions of carbon into the atmosphere. The carbon cycle is a broad, intricate system of carbon production and assimilation that includes both natural and anthropogenic processes. In nature, there are three main carbon "sinks" or repositories. These include the earth's atmosphere, oceans, and terrestrial ecosystem, which includes vegetation, and geological formations like fossil fuel reserves (Archer, 2011).

The Carbon dioxide in the atmosphere is on the rise. The trend of rising atmospheric Carbon dioxide over the years especially after industrialization is depicted in **Figure 1**.

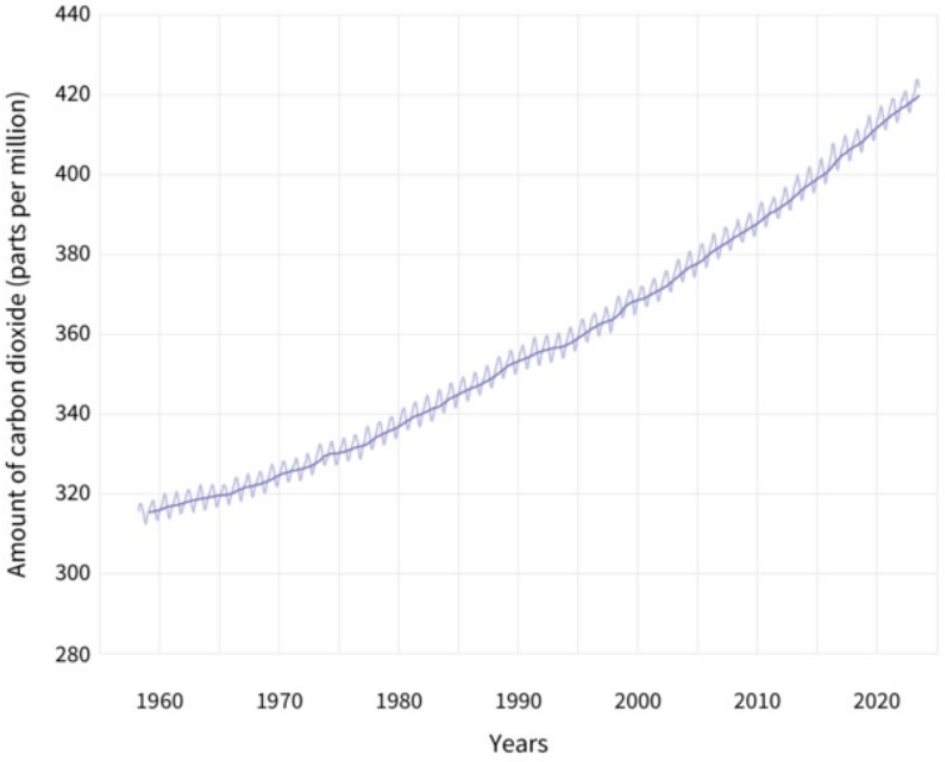


Figure 1: Trend of rising atmospheric CO_2 over the years. (Source: www.climate.gov)

The annual rate of increase in atmospheric Carbon dioxide from 1960 to 2020 is about 100 times greater than previous natural increases at the end of the last ice age, 11000 – 17000 years ago. In the 1960s, the global annual increase in atmospheric CO_2 was 0.6 ± 0.1 ppm, which in the last decade was closer to 2.3 ppm (Dlugokencky *et al.*, 2018). It is estimated that the global average atmospheric Carbon dioxide was 417.06 ppm in 2020-2022. It is reported that the increase in atmospheric Carbon dioxide over a short period from 2021 to 2022 was 2.13 ppm (Lindsey, 2020). According to him, the projected concentration of CO_2 in the atmosphere may vary from 540 ppm to 970 ppm in 2100, compared to 280 ppm in the pre-industrial era and 416.82 ppm in 2020. The studies carried out by Davis (2017), established the role of atmospheric Carbon dioxide in attributing to global warming. Atmospheric Carbon dioxide and other greenhouse gases which get concentrated in the atmosphere by the combustion of fossil fuels and unscientific land use practices capture infrared energy radiated from the surface of the Earth, resulting in the greenhouse effect and thereby global warming. The recent report of the Intergovernmental Panel on Climate Change (IPCC, 2018) set forth the urgent

need for keeping global warming levels below 1.5°C to circumvent the upcoming issues in the area of climate change.

According to Assessment Report 6 of the IPCC, a rise of 1.07 °C from 1850 to 2019 is estimated and this temperature rise is mainly due to anthropogenic factors (SAPCC, 2022). It is reported that even with an increase of 1.5°C temperature, there is an increase in the heat waves and temperature which becomes more severe at 2°C of warming. The Conference of the Parties (COP 26) which took place in 2021 in Glasgow agreed to limit the temperature rise to 1.5°C. Even an increase in temperature to 1.5°C led to an increase in sea level, extreme climate events, and bleaching of coral reefs. The COP 27 which was held in Egypt in November 2022 decided to limit the temperature rise to 1.25°C (https://www.ipcc.ch/assessment-report/ar6/).

In response to the increase in atmospheric Carbon dioxide levels, concentrations of CO_2 in the oceans are also increasing leading to a decrease in oceanic pH (Harrould Koleib and Herr, 2012). The effects of an increase in Carbon dioxide in the atmosphere lead to climate change, which results in the melting of ice and ocean warming, together with heat waves, extreme rainfall events, wildfires, disease outbreaks, and threats to food and water security (Mahato, 2014).

Consequent to policy interventions, greenhouse gas emissions are to be reduced by 7% each year until 2030 to achieve the target. Net zero emissions of greenhouse gases are also proposed in the summit along with interventions for reducing deforestation and promoting afforestation. Climate change mitigation and adaptation are to be planned to achieve net zero emissions by 2070 (Muller *et al*., 2020). To practice net zero emissions of CO_2 there should be a balance between the absorption and emission of CO_2 which can be maintained by a sufficient afforestation program (https://www.ipcc.ch/assessment-report/ar6/). As trees act as a sink to absorb CO_2, Carbon offset planting is one of the methods that helps in the reduction of greenhouse gases in the atmosphere (Jana *et al*., 2010).

Figure 2 depicts the Representative Concentration Pathways (RCPs) which are trajectories of greenhouse gas concentration in the atmosphere adopted by the IPCC in their Assessment Report 5. The RCPs try to capture trends in the concentration of greenhouse gases in the atmosphere as a result of enhanced human activities in the

future. The numerical values for RCPs are 2.6, 4.5, 6, and 8.5 and they represent the radiative forcing values in the year 2100. RCP 8.5 would result in the highest greenhouse gas concentration whereas RCP 2.6 is the lowest concentration. RCP 4.5 is described as an intermediate pathway. The temperature rise projected by 2100 under RCP 8.5 is 4.9°C whereas for RCP 2.6 it is 1.5°C. RCP 4.5 is described as an intermediate pathway to limit temperature up to 2.4°C. For limiting the temperature up to 2.4°C many mitigation measures are to be planned to reduce Carbon dioxide emission. Afforestation in degraded forest areas and avenue tree planting in open spaces is an economic and nature-based solution to capture Carbon dioxide in the atmosphere (IPCC, 2018).

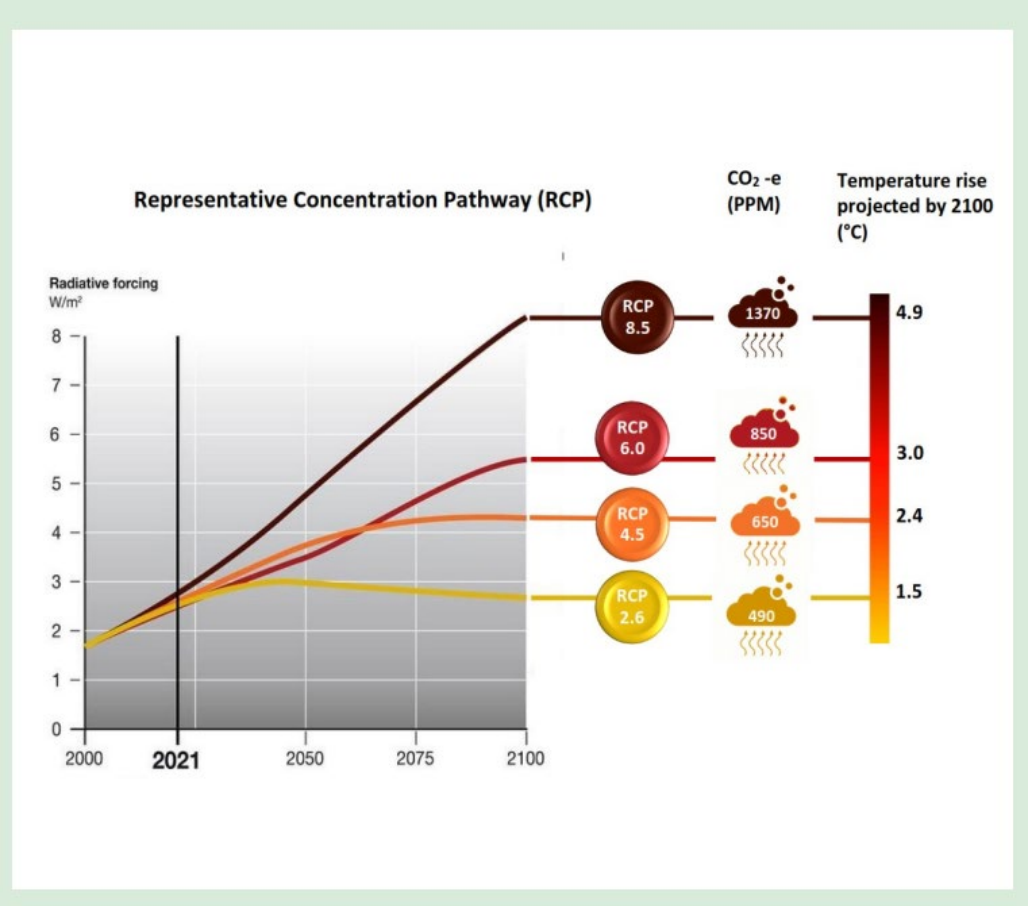


Figure 2: Representative concentration pathway (Source: IPCC, 2014)

Thus Carbon dioxide emission has to be reduced by following low carbon pathways including renewable technologies and carbon sequestration strategies. Carbon sequestration is the process of taking out carbon from various sources and depositing

it in long- or short-lived reservoirs (Nogia *et al*., 2016). Methods of carbon sequestration include agroforestry, wetland restoration, oceans as sinks, and geological injection. Agroforestry is an adaptive method as trees are natural sequesters of carbon and utilize it in the process of photosynthesis and store it in the form of biomass or wood. Wetland conservation is also an adaptive measure as wetland soil is an important natural carbon pool or sink. Wetlands conserve 14.5 % of the soil carbon found in the world. Oceans are also good sinks of Carbon dioxide. Similarly, geological formations capture Carbon dioxide. (Reddy *et al.,* 2022). Comparing various methods, carbon sequestration in terrestrial biomass is a meaningful and cost-effective approach for reducing the ill effects of climate change. Terrestrial ecosystems are key carbon sinks owing to the storage of Carbon dioxide in live and dead organic matter (Odiwe *et al*., 2016).

Photosynthetic assimilation of atmospheric Carbon dioxide by land plants offers one of the best methods of terrestrial carbon sequestration. Morphological and biochemical responses of plants to elevated levels of CO_2 vary depending on the physiology and anatomy of the plant. Planting fast-growing tree species to absorb excess atmospheric CO_2, an idea of carbon offset planting has gained potentiality, leading to the identification of tree species with high CO_2 sequestration capabilities. Several researchers have studied carbon sinks and sequestration of Carbon dioxide using vegetation cover. McPherson (1998) mapped Carbon dioxide storage and sequestration in biomass of the urban green cover throughout Sacramento County, California, and estimated that roughly 6 million trees of the Country absorb approximately 238000 tons of Carbon dioxide annually.

According to Kiran and Kinnary (2011), roadside trees in urban areas play a vital role in maintaining the ecological balance of a crowded and carbon-polluted environment created by the burning of fossil fuels. The study carried out along a 42.33 km stretch of road in Kolhapur city has revealed that the amount of carbon sequestered annually by the roadside trees is 54.36 tonnes (Desai and Nandikar, 2012). Scharenbroch (2012) stated that crucial traits for carbon sequestration and storage include a long life span, high wood density, and high tolerance to various urban stress factors. From an urban planning point of view, the development of a

low-carbon pathway is possible by managing urban green cover dynamics and species composition.

In the present study, an attempt has been carried out to assess the morphological and biochemical responses of selected tree species under an elevated supply of CO_2. The Carbon dioxide-controlled chambers are used to study the responses of tree species to enhanced levels of CO_2. The Carbon dioxide-controlled chambers used for the present study were cost-effective and replicable. Assessment of the carbon sequestration potential of the selected tree species was carried out and listed. The study provides insights for common people and policymakers to develop climate change mitigation strategies using the selected species.

OBJECTIVES

The present study has attempted to identify avenue tree species ideal for tropical climatic conditions by conducting Carbon dioxide sequestration studies using controlled growth chambers. The specific objectives outlined in the study are:

- Identification of avenue tree species ideal for tropical climatic conditions and collection of information on their natural mode of multiplication and growth.
- Multiplication in nurseries and maintenance up to desired stages of growth for experimentation along with standardization of growth conditions and acclimatization of characteristics.
- Conduct Carbon dioxide sequestration studies in selected plants using controlled growth chambers under varying concentrations of Carbon dioxide and other growth conditions along with an assessment of the changes in microclimatic conditions associated with the chamber brought about by the growth of plants under varying levels of Carbon dioxide supply.
- Assessment of the changes in growth, biochemical, and biomass content under varying levels of Carbon dioxide supply.
- Listing up of plants having higher Carbon dioxide sequestration potential and optimization of conditions of highest sequestration efficiency.

For a better elucidation of the above objectives, the results of the present study are depicted in two chapters. Chapter I deals with the identification of avenue trees, their nursery trials for multiplication, the design of experimental systems for phyto-

sequestration studies, the standardization of conditions, and the assessment of changes in microclimatic conditions attributed by the plants in response to varying influx of Carbon dioxide. Chapter II deals with an evaluation of the growth and biochemical responses of selected tree species, subjected to varying levels of Carbon dioxide.

CHAPTER I

EXPERIMENTAL SYSTEMS FOR PHYTO-SEQUESTRATION STUDIES AND STANDARDIZATION OF MICROCLIMATIC CONDITIONS

1.1 INTRODUCTION

A wide range of Controlled Environmental Systems (CES) have been developed for carbon sequestration studies worldwide. The purpose of these systems includes monitoring the CO_2 flux, and evaluating the microclimatic conditions like temperature, humidity, light intensity, etc., within the chambers when the plants are grown under controlled environmental conditions (Dias Carlson *et al*., 2018). By providing clearly defined environments for plant growth and development, CES like growth chambers and greenhouses offer the potential to accelerate scientific progress. In some modern growth chambers, especially for carbon assimilation studies, Carbon dioxide control or monitoring equipment is designed as a standard feature. Good control facilities in the chamber help to moderate extreme Carbon dioxide build-up or depletion. Day and night assimilation of plants inside the chambers can be continuously monitored using these controlled chambers. They help the researchers study the growth, morphological, and biochemical responses of plants under specific growth conditions and time intervals. Also, there are differences reported between plants grown in open fields and those in controlled growth chambers. Certain parameters that are studied using controlled chambers cannot be studied in an open-field experiment, and this makes the study using controlled chambers more relevant. However, field chambers and open-air releases are still been used for investigating the plant responses to various gaseous inputs under real-world conditions (Sionit *et al*., 1981).

Controlled growth chambers help in assessing the long-term effects of CO_2 enrichment on selected plants for their entire life cycle (Davidson *et al*., 2016). Such systems can also be used for monitoring the changes at specific stages of plant growth. In addition to conventional growth chambers and greenhouses, CES such as phytotrons, portable growth chambers, and sunlit controlled environment chambers are commonly in use in recent times. Open-top chambers help in studying the physiological responses of plant species to elevated CO_2 (Wang *et al*., 2019). One of the objectives of the study is to conduct Carbon dioxide sequestration studies in

plants using Carbon dioxide-controlled chambers under varying concentrations of Carbon dioxide. Microclimatic conditions associated with the chambers are also monitored along with the growth and biochemical responses of plants under elevated CO_2. The state-of-the-art research undertaken in this area is outlined below.

1.2 REVIEW OF LITERATURE

1.2.1 Controlled Environment Systems

Over the years a wide range of methodologies have been adopted to understand the responses of tree species to elevated CO_2. In the earlier stages, studies were carried out mainly using greenhouses, glass houses, and leaf chambers and later using phytotrons or other controlled environment chambers, or their modifications. In all these methods, an artificial environment is created instead of a natural ecosystem condition. A more refined experimental setup evolved later, which permitted interaction with more natural environmental conditions which included open-top chambers (OTC), free air Carbon dioxide enrichment systems (FACE), and screen-aided Carbon dioxide control (SACC) systems (Machacova, 2010).

Carbon sequestration studies using growth chambers were undertaken by Mousseau and Enoch (1989), and Fabreguettes *et al*. (1992). The experimental growth chamber of Mousseau and Enoch (1989) was 1.0 m in height with a ground area of 2.0 m^2. The chamber was built using a 28m transparent polypropylene sheet that was glued to an aluminum frame. Pure CO_2 was trickled into the air stream of the CO_2-enriched growth compartment at a constant flow rate. About 350 and 700 ppm of CO_2 were retained in the CC and TC, respectively. Throughout experimentation, the CO_2 concentration was measured using an infrared gas analyzer calibrated with bottled calibration gases. The air movement inside the chamber was observed. The growth chamber under experimentation by Fabreguettes *et al*. (1992) had a height of 1.3m and a ground area of 1.0 m^2. Photosynthetic photon flux density maintained in the chamber was 800 μ mol/m^2/s. Carlson and Bazzaz (1980) reported the use of inexpensive growth chambers that could be moved out of greenhouses and used with natural light. Davidson *et al*. (2016) experimented in a conventional growth chamber for 38 days using *Prunus persica*, in which the ambient CO_2 concentration was retained at 400, and the elevated CO_2 at 800 μ mol/mol.

Greenhouses had structural frames covered with glass, fiberglass, polyvinyl chloride, polyethylene, or other transparent materials that permit plants to grow in a controlled environment. They are equipped with heaters and ventilation systems. The fans inside the greenhouses were operated continuously to ensure adequate mixing of the air and CO_2. The advantage of a greenhouse over an open field is that temperature can be controlled in the greenhouse, whereas in the field, it is difficult. High humidity and low windspeed are normally maintained inside greenhouses (Drake *et al*., 1985). Also, low light intensity can be provided inside the greenhouses, as they transmit only two-thirds to three-fourths of sunlight. Light quality and intensity in greenhouses approximate the natural level of a controlled environment. Sunlight is allowed to enter and trap heat inside the chamber and create a warm and humid environment whereas a phytotron is a more refined version of a greenhouse, which regulates lighting, temperature, humidity, atmospheric gases, and soil nutrients and possesses a mechanism to monitor the growth of plants. A wide variety of plant species can be accommodated by phytotrons, which also encourage diverse environmental conditions. Several growth chambers and greenhouses are organized in a phytotron facility so that different environmental factors can be stimulated for conducting simultaneous studies.

For a study, *Arachis hypogea* was grown in greenhouses with ambient (360 µmol/mol) and elevated (720 µmol/mol) CO_2 concentrations. Proper facilities for airflow and temperature were provided. The ventilation fan speed and electric heaters were controlled by a microprocessor algorithm (Vu, 2005). Epron *et al*. (1996) used the same principle that elevated CO_2 concentrations in the greenhouse were established by injecting pure industrial CO_2 at the blower inlet at a constant flow rate of 2 dm^3 min^{-1}. The concentrations of atmospheric CO_2 in this study were ambient and ambient + 350 ppm (elevated). The CO_2 enrichment was initiated when the saplings were transplanted and were maintained day and night for 20 months with regular monitoring with an infrared gas analyzer. The experiments of Lahive *et al*. (2018) were conducted within four compartments in a 2 × 2 square arrangement of a greenhouse suite designed to study the effects of climate change on *Theobroma cacao*. Two compartments were maintained at elevated CO_2 (700 ppm) and two at ambient CO_2, each treatment being represented on either side of the square. The CO_2 enrichment system was controlled by a centralized computer system. In all

compartments, a wall-mounted infrared gas analyzer was installed which continually measured the CO_2 concentration of the air within each compartment. The CO_2 concentration in the elevated CO_2 compartments was set to 700 ppm. For CO_2 enrichment, the flue gas from each compartment's natural gas burner was used.

The Field Tracking Chamber consisted of a polyvinyl chloride tubing frame with plastic sheeting sealed to a galvanized metal frame. Air temperature within the chamber was maintained at the desired ambient level (Drake *et al*., 1985). The mass of CO_2 going into the chamber was calculated from the flow rate and the time that CO_2 was injected. Fans inside the chamber helped to guarantee proper mixing of the air and CO_2. They were used over natural vegetation. Field tracking chambers were used to study canopy and ecosystem responses to a combination of variable and controlled-field environments (Allen *et al*.,1992). Open-top field chamber was a type of field tracking chamber. Plant exposure unit affects the studies in the field (Heagle *et al*., 1979) in an open-top field chamber. The chamber helps in exposing both row crops and plants in pots to gaseous pollutants.

Leaf chambers help to control the environment around the leaf. Water vapor loss and CO_2 assimilation can be measured using a leaf chamber. The leaf gas exchange system by Sinclair and Allen (1982) would be well adapted for studying the effect of prolonged exposure to elevated CO_2 levels on photosynthesis. This system was capable of field operation and was able to track environmental temperature, humidity, and solar radiation, along with continuous measurement of both water vapor, CO_2 exchange, and control of CO_2 concentration. The leaf chamber was made up of two chrome-plated brass rings separating two transparent Teflon discs. On each ring, the leaf was placed between rows of monofilament lines (Drake *et al*., 1985). Leaf cuvettes are used to control the environment around the leaf. Cuvette designs for leaf gas exchange measurements have been proposed by various authors like DeJong *et al*. (1982); Field *et al*. (1982); Huck *et al*. (1983); and Valle *et al*. (1985). The simplest leaf cuvette systems have measured only CO_2 assimilation. Water vapour loss and CO_2 assimilation are measured to evaluate the effect of elevated CO_2 treatment on the supply of CO_2 through stomata to intercellular spaces (Allen *et al*., 1992). For measuring the exchange of gas between leaves and their surroundings, closed and open cuvette devices are employed.

Portable growth chambers are designed to utilize the sunlight available in the greenhouse along with other sources of light. They are assumed to be low-budget approximations of greenhouses (Drake *et al*., 1985). The chamber described by Carlson and Bazzaz (1980) is made of glass and wood. Sorption of gases like CO_2 and water vapour is minimized on interior wood surfaces. A wheeled frame of steel supports this chamber. A fan in the plenum circulates air across heat exchangers and back into the growth chamber through a bottom vent. Valves and flowmeters were used to sample the air from the chambers.

Reicosky (1990) measured canopy evapotranspiration using the Portable field chamber technique. This technique has also been applied to canopy photosynthetic CO_2 exchange measurements in subsequent studies. A fan was used to circulate air within the transparent chamber. The portable field chamber created in Florida (Jones *et al*., 1982; Zur *et al*., 1983; Boote *et al.*, 1984) included a mirror-type hygrometer for detecting humidity and a nondispersive infrared gas analyzer for measuring Carbon dioxide. Portable field chambers are used to measure canopy photosynthesis and transpiration rates (Allen *et al*., 1992).

Sunlit-controlled environment chambers are based on a closed-loop air circulation system with computer-managed environmental controls. They have walls constructed of polyester film. The photosynthetic CO_2 exchange rate is measured using this chamber, along with the rates of transpiration (Musgrave and Moss 1961; Moss *et al.*, 1961; Baker and Musgrave 1964, Egli *et al.*, 1970). The SPAR chamber system described by Jones *et al.* (1984) provided measurements of canopy photosynthetic CO_2 exchange rate and transpiration rate at 5-minute intervals. These chambers include sensors that control temperature, humidity, and CO_2 as well as feedback systems like thermostats and temperature-controlling equipment like heaters to maintain the desired temperature. The interaction of CO_2 with temperature, humidity, and light intensity was studied. Sunlit controlled-environment chambers are used to study canopy and ecosystem responses to a combination of variable and controlled-field environments (Allen *et al*., 1992).

Phytotrons are integrated collections of controlled growth facilities. They are the most complex form of controlled environment facility. A wide range of

environmental factors can be studied simultaneously using phytotrons. Phytotrons are used to investigate how the environment controls and modifies plant growth and development. They played an important role in certain phases of ecological research. Phytotrons are different from greenhouses or growth chambers as they have controlled environmental factors. Photosynthetic Proton Flux Density (PPFD) available in the growth chamber is 300-700 μmol/m^2/s. Most plants require high photosynthetic energy to adapt to elevated levels of CO_2. High-intensity discharge lamps provide the PPFD required in CO_2-controlled chambers (Lewis *et al*., 1996). The advantage of phytotrons over field experiments is that only selected variables are controllable (Drake *et al*., 1985). Phytotrons are constructed with compressors, pumps, and valves where only selected variables are controllable (Drake *et al*., 1985).

In the experimental design of Sionit *et al*. (1981), CO_2 was injected into the chambers automatically and its concentration was monitored using an infrared gas analyzer. The injection system provided CO_2 concentrations of 450 + 40, 675 + 20, or 1,000 + 10 ppm in three different chambers. The experiment was set up in a split-plot design with four replications.

Plants were continuously exposed to CO_2 concentrations of 350 or 1000 ppm CO_2 in controlled growth chambers in Phytotrons where the concentration of CO_2 was automatically monitored and controlled (Sasek *et al*.,1985). A major advantage of the phytotron was that multiple chambers or rooms may be used to create matrices of environmental variables (Allen *et al*., 1992). Phytotron chambers were used to obtain multiple-factor controls and to gain the space required for larger experiments (Allen *et al*.,1992).

There are advantages and disadvantages to the various methods discussed above. The suitability of a controlled environment chamber depends on the specific needs of the researcher. If a particular aspect is focused on the experimental study, a growth chamber is preferred to other methods. Phytotrons can generate and control many desired environmental conditions and also help in repeating the experiments. To understand the photosynthetic and respiratory aspects of plants, a leaf chamber is more advantageous (Strain and Cure, 1985).

1.2.1.1 Open top chambers (OTC)

Typically, open-top chambers are composed of metal structures with frustums on top and clear vertical side walls made up of polyvinyl chloride or plexiglass. Air can circulate through an opening in the center of the frustum to lessen the effects of temperature and humidity in the chamber. Carbon dioxide-enriched air is circulated using a tube and the distribution of CO_2 within the chamber is assured by air blowers (Leadley *et al*., 1997; Machacova, 2010).

Different experimental models and designs are used for Carbon dioxide enrichment experiments on tree species by various authors. The experimental designs and microclimatic environments maintained in OTC experiments by various authors are provided in **Table 1.2.1**.

Table 1.2.1: Experimental models & designs used for CO_2 enrichment experiments on trees

Sl. No.	Author and year	Experimentation system	Experimental design	Microclimatic environment
1.	Radin *et al*., 1988	Open top chamber	The base area of the chamber- $9m^2$	Ambient CO_2- 356µL/L Elevated CO_2- 643 µL/L
2.	Drake *et al*., 1989	Open top chamber	Diameter- 0.8 m Height- 1 m. 3 sections of the chamber- lower plenum, main chamber, frustum. Made with PVC frame.	Ambient CO_2- 350 µL/L Elevated CO_2 – 686 µL/L
3.	Bhattacharya *et al*., 1990	Open top chamber	The chamber is made of polythene sheet	Ambient CO_2- 356µL/L Elevated CO_2- 666 µL/L
4.	Sanders *et al*., 1991	Open top chamber	Diameter- 3.1m Height- 2.4 m. The chamber is fitted with PVC-covered 45° frustum.	Temperature- 0.8°C higher inside the chambers compared with ambient air.
5.	Norris *et al*., 1996	Controlled ventilation open-top chamber (CVOTC)	CVOTC is made of polythene sheet. It consists of a ventilation unit and a chamber linked by air ducting. The chamber frame is made of galvanized steel. Frustum with 0.5 diameter reduced wind incursion.	Temperature- 1.6°C above ambient. Airspeed- 1m/s

6.	Rudorff *et al*., 1996	Open top chamber	Diameter- 1.5-4.5m, Height-2 to 2.5m. The chamber is composed of a metal frame covered by transparent plastic film.	Ambient CO_2- 350 µL/L, Ambient +150 µL/L.
7.	Rey and Jarvis (1997)	Open top chamber	3 treatments Ambient, Elevated, and Control (outside the chamber), and 18 trees present. The chamber is made of a cylindrical steel frame with polyethylene film.	Ambient CO_2- 350 µ mol/mol Elevated CO_2- Ambient + 350
8.	Van Oijen *et al*., 1999	Open top chamber	12 OTC was used for the experiment. OTC is hexagonal. Chamber walls are made of polycarbonate.	Temperature- 16.2 °C. Ambient CO_2 – 365 to 380 ppm in both the years. Elevated CO_2 – 716 to 720 ppm (1995) and 751 to 756 ppm (1996)
9.	Centritto *et al*., 1999	Open top chamber	3 treatments Ambient, Elevated, and outside blocks	Ambient CO_2- 350 µ mol/mol Elevated CO_2- Ambient + 350. PPFD- Above 1700 µ mol/m^2/s
10.	Janous *et al*., 2000	Open top chamber	Diameter of chamber- 2.5 m Height – 6 m. The perforated polyethylene duct is located on the inner side of the chamber.	Temperature inside OTC- 1.3 °C higher than outside.
11.	Bunce (2001)	Open-topped clear acrylic chambers	Height of OTC -1.8 m. 6 OTC present.	2 chambers with CO_2 concentration- 300 µ mol/mol Other 2 chambers- 600 µ mol/mol
12.	Coley *et al*., 2002	Open top chamber	Height of OTC -2.5 m. Constructed with an aluminum frame covered with plastic film.	Ambient CO_2- 300 to 400 ppm Elevated CO_2- 400 ppm above ambient.
13.	Aidar *et al*., 2002	Open top chamber	OTC made of aluminum and plastic	Ambient CO_2- 360 ppm Elevated CO_2– 720 ppm
14.	Katny *et al*., 2005	Open top chamber	Height of OTC- 2.4 m. Diameter -3.14 m. Duration of experiment- 5 weeks.	Ambient CO_2- 400 ml/L, Elevated CO_2- 720 ml/L
15.	Vanaja *et al*., 2006	Open top chamber	OTC fabricated with GI pipe and covered with PVC sheet.	Transmittance of light- 90%

16.	Netten *et al*., 2008	Open top chamber	OTC is made of fiberglass which helps in the low transmittance of infrared and high solar transmittance of visible light.	Increase in temperature in Elevated chamber- +2.3°C
17.	Moutinho Pereira *et al*., 2010	Open top chamber	Duration of the experiment- 3 growing season	Ambient CO_2- 365 ppm Elevated CO_2- 500 ppm PPFD -> 1000 µmol/m^2/s
18.	D'Andrea and Rinaldi, (2010)	Open top chamber	Closed systems (greenhouses, growth chambers, tunnels, closed top chambers), semi-open (open top chambers= OTCs), and open systems (Free-Air Carbon dioxide Enrichment facilities=FACEs)	Material used for construction of OTC- Fibreglass, polycarbonate, Plexiglass.
19.	Molau (2010)	Open top chamber	OTC is hexagonal and made of polycarbonate.	85% solar transmittance in visible wavelength
20.	Karowe and Grubb, (2011)	Open top chamber	The volume of chamber- 120.5 m^3	Ambient CO_2- 379 ppm Elevated CO_2 – 744 ppm
21.	Al Rawahy *et al*., 2013	Open top chamber	Duration of experiment- 60 days. Chambers fed with charcoal-filtered air. Ventilation rate- 45 m^3/min	Temp- 22 to 29°C Hum- 48 to 74% CO_2- 350,400, 450 ppb
22.	Messerli *et al*., 2015	Open top chamber	Height of OTC-1m. 8 hexagonal OTC present. 4 with elevated CO_2 and 4 with ambient CO_2.	CO_2 concentration- 600 µ mol/mol
23.	Chakraborty *et al*., 2015	Open top chamber	The experiment was a (2 × 2) factorial experiment of two cultivars of Brassica.	Ambient CO_2- 390 µ mol/mol Elevated CO_2- 550 µ mol/mol. Temp- 11 to 25° C. Hum- 80 to 85%.
24.	Pal, 2015	Open top chamber FACE- (Free Air Carbon dioxide Enrichment) ET AND CGT	OTC is made of polycarbonate sheets. The sealing of OTC is attached using aluminum angles. GI pipes are used for its manufacture. Height- 1.5 m, Wide-	Light transmission level- 80 to 85 %. Equipment for monitoring and controlling CO_2 in FACE is automatic. Polycarbonate sheets

		(Elongated tunnel and CO_2 gradient tunnel)	1.2 m, Length- 60 m.	provide an airtight atmosphere within the chambers.
25.	Janani *et al*., 2016	Open top chambers	4 treatments and 4 chambers are present. Dimension of OTC- 3×3×3 m. Chambers with GI pipe frame and covered with a polythene sheet. Duration- 125 days	Ambient CO_2- 380 ppm Treatment 2- 600 ppm, Treatment 3- 900 ppm, Treatment 4- 900 ppm maintaining temperature of ambient +4°C. Control- 380 ppm with no chambers
26.	Wang *et al*., 2019	Open top chamber	Height of OTC- 3 m. Diameter of OTC- 4m. 4 treatments and 12 OTC were designed.	Elevated CO_2- 700 µ mol/mol

In the open-top chamber designed by Drake *et al*. (1989), a frustum was incorporated to lessen the incursion of air into the open-top chambers during windy conditions. The diameter and height of the chamber were 0.8 m and 1.0 m respectively. Chambers were built in three sections: a lower plenum, a main chamber, and a frustum. Air was introduced and mixed in the chambers by two squirrel-cage blowers for air circulation within the chamber. A small amount of pure CO_2 was injected into the air stream at the remote blower to elevate the CO_2 concentration. Air from each chamber was continuously sampled by a pump located near the chamber in the field. Water vapour was purged from the sample before injection into the gas analyzer. CO_2 concentrations in these air samples were measured automatically in each chamber every 15 min using an infrared gas analyzer and data acquisition computer. According to Drake *et al*. (1989), the open-top chamber system has been used in climate change studies because the construction costs of the OTC systems were low in comparison to closed-top chambers (CTC) and free-air CO_2 enrichment systems.

The Open-top field chambers developed by Bhattacharya *et al*. (1990) were open-ended cylindrical baffles with a diameter of 3m and a height of 2.4m. They were constructed using aluminium frames covered with PVC plastic films. There was a 45-degree frustum to narrow the upper opening. The inside wall was perforated and served as a duct to distribute air uniformly into the chamber. The axial fan was used to supply air to this duct. The Infrared gas analyzer (IRGA) was used to measure

CO_2 concentrations in the chamber which was based on the principle that CO_2 absorbs infrared energy.

Sanders *et al*. (1991) used open-top chambers with a diameter of 3.1 m and height of 2.4 m fitted with a PVC-covered 45° frustum restricting the size of the open top to 2.2 m. The ventilation was provided by an axial fan giving an air flow of $1m^3/s$. The open-top chamber designed by Molau (2010) was hexagonal-shaped and made up of polycarbonate sheets with 85 percent solar transmittance. Radin *et al*. (1988) developed an open-top chamber with a basal area of $9m^2$ surrounded by a transparent plastic film mounted on a wooden frame.

In the experimental study, Norris *et al*. (1996) developed controlled-ventilation OTC (CVOTC) in such a way as to reduce Carbon dioxide consumption by a combination of automatically controlled ventilation and recirculation of the chamber air and it was capable of being operated at remote sites. A motorized baffle aided in the recirculation of air and ventilation. The microcomputer-based control system of novel design continuously adjusted the rate of Carbon dioxide injection, motor power, and baffle position. The chamber was made of polythene sheet and the frame consisted of standard galvanized steel tubing. In contrast to glass, plastic film cladding was used which prevented damage from hail.

The open-top chamber developed by Rudorff *et al*. (1996) was cylindrical with a diameter of 1.5m to 4.5m and a height ranging from 2 to 2.4m. They were composed of a metal frame covered with a transparent plastic sheet. Air was usually forced into an OTC employing an axial fan positioned outside the chamber and a pipe moves the air from the fan into the OTC. The inside wall of OTC had hundreds of circular holes to uniformly distribute air throughout the vegetation.

Rey and Jarvis (1997) designed the experimentation in such a way that there were 3 treatments, 6 trees in ambient CO_2 concentration (350 µmol/mol), 6 in elevated CO_2 concentration (Amb + 350 µmol/mol), and other 6 trees were grown outside chambers to assess the chamber effect (Control treatment). The open-top chambers consisted of a cylindrical steel frame covered with polyethylene film. CO_2 supply was maintained day and night throughout the year. CO_2 concentration was kept at a target value of ± 50 µmol/mol. The average temperature increase was 1.2° C. Light was attenuated by 10 % by the walls of OTC.

In the experimentation by Van Oijen *et al*. (1999), OTC was hexagonal. Chamber walls were made of 3 mm polycarbonate, which was 88% transparent to photosynthetically active radiation. The system consisted of a cooling tank. The cooling system reduced the temperature in the OTC.

In the experimentation setup of open top chamber by Centritto *et al*. (1999) for studies in *Prunus avium,* ambient CO_2 concentration was 350 µ mol/mol, and elevated was CO_2- Ambient + 350. Photosynthetic proton flux density (PPFD) was above 1700 µ mol/m^2/s.

Long-term effects of elevated CO_2 (EC) and air temperature on the growth and physiology of tree species were studied by Kellomaki *et al*. (2000). 4 treatments 1) Ambient temperature and CO_2 concentration 2) Doubled Ambient CO_2 (EC) 3) Elevated ambient temperature (ET) and 4) EC and ET were maintained. 16 individual chambers with 16 trees inside the chambers and 4 trees outside (under ambient conditions) were present. The chamber was of a cylindrical structure with 8 walls. The walls were constructed from 12 pieces of double-wall glass and acrylic sheets. The chamber had a conical roof consisting of 8 acrylic plates, to allow the precipitate to run off.

Bunce (2001) used an open-top-clear acrylic chamber, having coverage of 1.1 m^2 of the ground. The height of the chamber was 1.8 m. Carbon dioxide was introduced into four of the chambers at the inlets of mixing fans. Two chambers had a CO_2 concentration of 300± 50 µ mol/mol and the other two chambers had 600± 50 µ mol/mol.

Open top chambers of Aidar *et al*. (2002) were constructed with aluminium and plastic. Air sampling and automated measurements were performed at 5-minute intervals throughout the experimental period. CO_2 concentration in the CC and TC were maintained at 360 ppm and 720 ppm respectively.

In the study by Karowe and Grubb (2011), the volume of the open-top chamber was 120.5 m^3. The ambient CO_2 concentration was 379 ppm. 12 chambers were maintained at an elevated CO_2 concentration of 744 ppm. CO_2 levels were

monitored using an infrared gas analyzer and the flow of CO_2 was regulated using a flow meter.

Open-top chamber by Coley *et al*. (2002) was 2.5 m tall and constructed with an aluminium frame covered with clear plastic film. Ambient chambers were maintained at 300- 400 ppm of CO_2. Elevated CO_2 chambers were maintained at 400 ppm above the ambient concentration. The PPFD reached up to 2000 µmol/m^2/s.

In a study by Katny *et al*., (2005), the plants were exposed to 5 weeks of CO_2 supply with an ambient CO_2 concentration of 400 and elevated concentration of 720 mL/L in circular open-top chambers of 3.15m diameter and 2.40m height. PAR conditions, air temperature, and relative humidity were recorded inside the OTC continuously and reported on an hourly basis during the period of exposure.

The OTC employed by Vanaja *et al*. (2006), consisted of galvanized iron (GI) pipe, covered with a PVC sheet of 120-micron gauge allowing 90 % transmittance of light. To lessen the dilution impact of air current within the chamber, a frustum was positioned at a specific height from the chamber. A CO_2 analyzer, pump to remove the sample from OTC, valves, and meters to control and regulate CO_2 and airflow, and CO_2 gas cylinder for the supply of CO_2 gas, along with PLC and SCADA monitor to control the desired CO_2 levels, were parts of the facility. These facilities aid in the continuous monitoring of the CO_2 concentration, temperature, and humidity associated with each OTC.

Netten *et al*. (2008) employed OTC made with fiberglass, which helped in the low transmittance of infrared and high solar transmittance of visible light. The sides of the chamber were inclined inwards to trap more heat.

The experimental setup of Moutinho Pereira *et al*. (2010) consisted of OTC with polyethylene film with a 75% light transmittance. For monitoring the climate variables inside and outside of the OTC, sensors were connected to a logger from delta T devices.

In the experimental model of D'Andrea and Rinaldi, (2010) the OTC was double-walled, with the inside wall perforated and served as a duct helping in the uniform distribution of air throughout the chamber. Quantification of the airflow rate was

difficult due to the complex nature of the air management system. However, OTC helped in controlling climatic conditions in a closed environment.

Karowe and Grubb (2011) used an open-top chamber with a volume of 120.5 m^3. The ambient CO_2 concentration was 379 ppm. Twelve chambers were maintained at an elevated CO_2 concentration of 744 ppm. The CO_2 levels were monitored using an infrared gas analyzer and the flow of CO_2 was regulated using a flow meter.

The open-top chambers used by Al Rawahy *et al*. (2013) were equipped with a diurnal cycle of 12 hours of light, and the temperature inside the chambers ranged from 22°C (min) to 29°C (max), relative humidity between 48% and 74%, CO_2 supply in the range of 350 ppb, 400 ppb, and 450 ppb, and a ventilation rate of 45 m3/min. These conditions were designed to simulate the projected effects of climate change on plant growth and development.

In the study of Messerli *et al*. (2015), eight hexagonal OTC (4 with elevated CO_2 and 4 with ambient CO_2) each with 1.2 m^2 of the ground area were built. The height of each chamber was 1.0m. Each OTC had its ventilation system consisting of a fan placed in a mixing box. Here the air was pushed into the OTC at a flow rate of 5.66 m3 min^{-1}. The CO_2 sensor, which was installed at the end of the pipe attached to the plastic box, was also protected by the flexible PVC pipe. Recirculating the air from the chamber decreased CO_2 usage and kept CO_2 levels steady. Each OTC with elevated CO_2 had its CO_2 control system, which was fixed inside a PVC electrical box and included a sensor transmitter, a power source, a transformer, and a solenoid valve. Each OTC with elevated CO_2 was operated in parallel and each had its CO_2 supply, to easily monitor the CO_2 consumption in each chamber.

Two cultivars of Brassica were experimented in an open-top chamber by Chakraborty *et al*. (2015). The ambient CO_2 concentration in the chamber was 390 µ mol/mol and the elevated CO_2 concentration was 550 µ mol/mol. Temperature and humidity in the chambers varied from 11 to 25° C and 80 to 85%.

Simulation and modeling of climate change studies were undertaken by Pal (2015). Open top chamber designed for this purpose included polycarbonate sheets with a light transmittance level of 80 to 85 %. Sealing of OTC was achieved using aluminium angles. GI pipes were used for the construction of the FACE ring. ET

and CGT (Elongated tunnel and CO_2 gradient tunnel) systems were developed and the chamber was maintained at 1.2 m wide and 1.5 m in height. Polycarbonate sheets provide an airtight atmosphere within the chambers. Measurements of wind direction, wind velocity, and CO_2 were undertaken by a computer-controlled system to adjust the CO_2 flow rate.

The experimental setup of Janani *et al*. (2016) was similar to that of Vanaja *et al*. (2006), where the CO_2 levels were monitored through SCADA and PLC. The open-top chambers were of dimensions 3×3×3 m fabricated with GI pipe and covered with PVC sheet. Four treatments namely ambient (380 ppm), treatment 2 (600 ± 50 ppm), treatment 3 (900 ± 50 ppm), and treatment 4 (900 ppm of CO_2) were maintained.

In the study of Wang *et al*. (2019), the open-top chamber was 4 m in diameter and 3 m in height with a 45° slopping frustum. Four treatments and 12 OTC with 3 replicas for each treatment were designed.

1.2.1.2 Free Air Carbon dioxide Enrichment (FACE)

Measuring the effect of elevated CO_2 using FACE is a more natural way of estimating how plant growth will change in the future as the CO_2 concentration rises in the atmosphere. FACE experiments are conducted on a wide range of plant species. In general, they constitute vertically oriented pipes that form a ring around the plot, that transfers and distributes Carbon dioxide. The dosage of Carbon dioxide is determined by the actual concentration of Carbon dioxide within the plot as well as other climatic elements like wind speed and direction. The supply valves are adjusted following the variations in wind speed and direction (Machacova, 2010).

The need to study the effects of CO_2 on vegetation in the natural field environment has led to the concept of artificially elevating the CO_2 by its release through a network of pipes. The history of the FACE method can be traced to various studies by agronomists (Baker and Musgrave, 1964; Allen *et al*., 1974; Harper *et al*., 1973). The face system used by Hendrey *et al*. (1999) employed feedback control technology to control the CO_2 in forest plots. CO_2 consumption by the FACE system was higher than open-top chambers on an absolute basis and it also helped to

investigate the long and short-term alterations within the entire forest ecosystem. Free-air CO_2 enrichment (FACE) allows open-air elevation of CO_2 without altering the microclimate. It was necessary to conduct FACE experiments in agricultural systems to evaluate the management and adaptation measures, such as finding genetic variation mechanisms in response to rising CO_2 and evaluating transgenic measures to increase yields and sustainability in predicted future atmospheres. (Ainsworth and Long, 2005).

The effect of FACE on the growth of Barley was studied by Manderscheid *et al*. (2009). The experiment was conducted in such a way that the fumigation treatments included two FACE circular experimental areas enriched with CO_2 of nearly 550 ppm and two control rings with ambient air of about 375 ppm. The FACE systems used by Castro *et al*. (2009) consist of four experimental blocks. The experimental systems included control (372 ppm) and enriched CO_2 (548 ppm) systems. Here compressed CO_2 was injected from fumigation pipes at supersonic velocity into the wind, thus making the surrounding air in a turbulent motion. A meta-analytic review study conducted in plants and ecosystems using FACE technology by Ainsworth and Long, (2005) described the experimental setup of FACE. Here the vegetation was exposed to 475- 600 ppm of CO_2. In this process, an array of horizontal or vertical vent pipes was used to release CO_2-enriched air. The first FACE systems utilized blowers or fans to inject CO_2-enriched air into the treatment area. A FACE approach has been used in more recent field investigations, in which emission tubes placed horizontally at the edge of a FACE octagon were used to generate high-velocity jets of pure CO_2 gas. One of the greatest drawbacks of FACE experiments was the very high cost arising from the high consumption of CO_2 during fumigation (Machacova, 2010). Since the wind has unrestricted access to the experimental plot, short-term CO_2 changes in FACE could be greater than those in OTC's trials. FACE allows studies that are more natural with an unaltered microclimate (Machacova, 2010). FACE eliminates the chamber effects, reducing solar radiation environment, unnatural wind flow, and turbulence (Drake *et al*., 1985). In FACE, interference of solar radiation and wind flow is low. Temporal variations in CO_2 concentrations, technical difficulties in installation, and design constraints for tall types of vegetation are the leading disadvantages when using FACE technology. Another disadvantage of the FACE experiment is the high cost due to the high consumption

of CO_2 during fumigation. Also, in FACE experiments, since the wind has free access to the plot, short-term CO_2 fluctuations are larger than in OTCs (Machacova, 2010).

1.2.1.3. Screen-Aided Carbon dioxide Control (SACC)

They are used to study the effects of elevated CO_2 on plants and ecosystems. It is similar to OTC in that it consists of a transparent screen that encloses a plot of vegetation. SAAC helps to reduce the microclimate effects associated with the OTC. The design of SACC is characterized by a transparent polycarbonate sheet mounted on a steel frame. Carbon dioxide is circulated within the plot through a pipe with small holes attached below the screen. A gap between the soil surface and the distribution pipe is maintained. The open top of the SACC allows the plot and the nearby field to equalize in temperature, air, humidity, and precipitation (Machacova, 2010).

Screen Aided Carbon dioxide Control (SACC) allowed temperature, air humidity, and precipitation to equalize between the plot and the surrounding fields (Rogers *et al*., 1983). According to Leadley *et al*. (1997), Screen-Aided CO_2 Control (SACC) technology, which was superior to OTCs in terms of its impacts on microclimate, requires substantially less CO_2 per experiment and each replication than FACE. Mixing the outside air with the CO_2-enriched air generates relatively uniform CO_2 concentrations. Screen-Aided CO_2 Control (SACC) system developed in this study ameliorated the microclimate problems associated with OTCs and lowered operating costs per experiment than FACE.

Upon comparison of the three Carbon dioxide-controlled systems, OTC can easily be employed to assess the effects of elevated CO_2 on individual tree species. Closed walls and frustum create an artificial microclimate within the chamber and hence microclimatic variations within the chamber can easily be monitored. FACE is a more advanced technique for subjecting plants to increased CO_2. It is closer to natural environment conditions compared to OTC. The CO_2 is released into the air around the plants by FACE systems using a series of pipes. The cost of FACE systems is more compared to OTC. It is a more sophisticated system with an unaltered microclimatic condition. One of the disadvantages of the system is that wind has free access to experimental plots and short-term CO_2 fluctuations are

larger. SACC systems were designed in such a way as to eliminate the disadvantage of both OTC and FACE. SACC systems use fans and screens in tandem to regulate the environment around plants. Systems like SAAC allow very precise environmental management around plants.

One of the main advantages of OTC is its cost-effectiveness. The design of OTC is significant as it influences the microclimatic conditions inside the chamber in response to plants and other experimental conditions. The nature and magnitude of materials used for the construction of chambers vary following the models proposed. Some of the prominent models are cuboidal, cylindrical, and hexagonal. The shape of the chamber has an influence on air movement and temperature distribution within the chamber. In most cases, the size and shape of OTC are dependent on the nature of experiments undertaken and the objectives proposed. Also, the materials used for the construction of OTCs include polythene sheets, polycarbonate sheets, and plastic films which permit sunlight to enter the chamber while preventing the escape of heat and gases outside. Other durable and comparatively less transparent materials such as aluminium, PVC sheet, fiberglass, and galvanized steel are also used for making OTC. Apart from these, the ventilation, irrigation, and microclimatic monitoring systems are also associated with different OTCs. All OTCs aim to provide a controlled environment for studies. The design of OTC is chosen based on the species' characteristics and specific research objectives.

In the present study, a modified version of the conventional open-top chamber is designed to assess the morphological, physiological, and biochemical responses of plants under elevated CO_2 conditions.

1.3. MATERIALS AND METHODS

As the study aimed to understand the changes in the microclimatic conditions inside the chamber along with the growth and biochemical responses of selected tree species under controlled conditions of CO_2 supply, the Carbon dioxide-controlled chamber is specifically designed for the purpose. Further details concerning the design and the mode of operation of the experimental system are detailed below.

1.3.1 Carbon dioxide- controlled chamber and experimentation process

The experiment was carried out in two controlled growth chambers, each with a volume of 6.32 m^3, constructed with PVC frames, and covered with 1 mm thick transparent Polyvinyl chloride sheets. The CC was equipped with the facility for the supply of ambient air through an air compressor, whereas the TC was equipped with the facility for the supply of CO_2-air mixture in specific doses. Both chambers were fitted with the facility for the analysis of CO_2 (ppm), temperature (^0C), and humidity (%). Apart from these, the chambers were fitted with an exhaust facility at the top for controlling the gaseous levels or microclimatic conditions inside the chamber, if required. Both the chambers were also fitted with a semi-automated facility for the irrigation of plantlets during experimentation. The schematic representation is depicted in **figure 1.3.1** and the experimental setup in **plate 1.3.1 (a, b and c**).

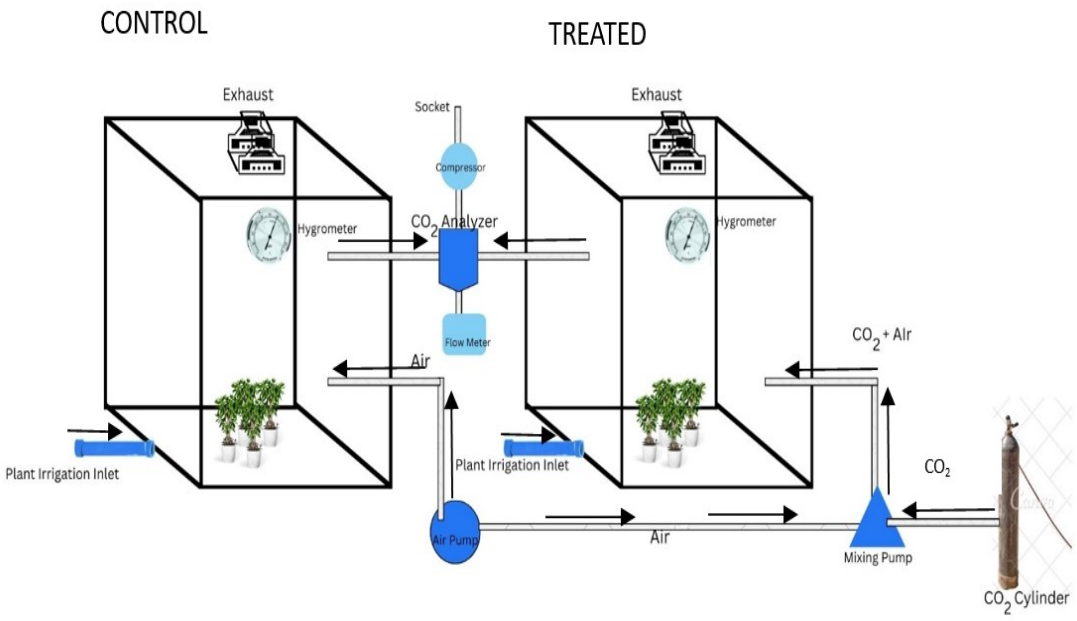


Figure 1.3.1: Schematic representation of Carbon dioxide controlled chambers (Control and CO_2-treated)

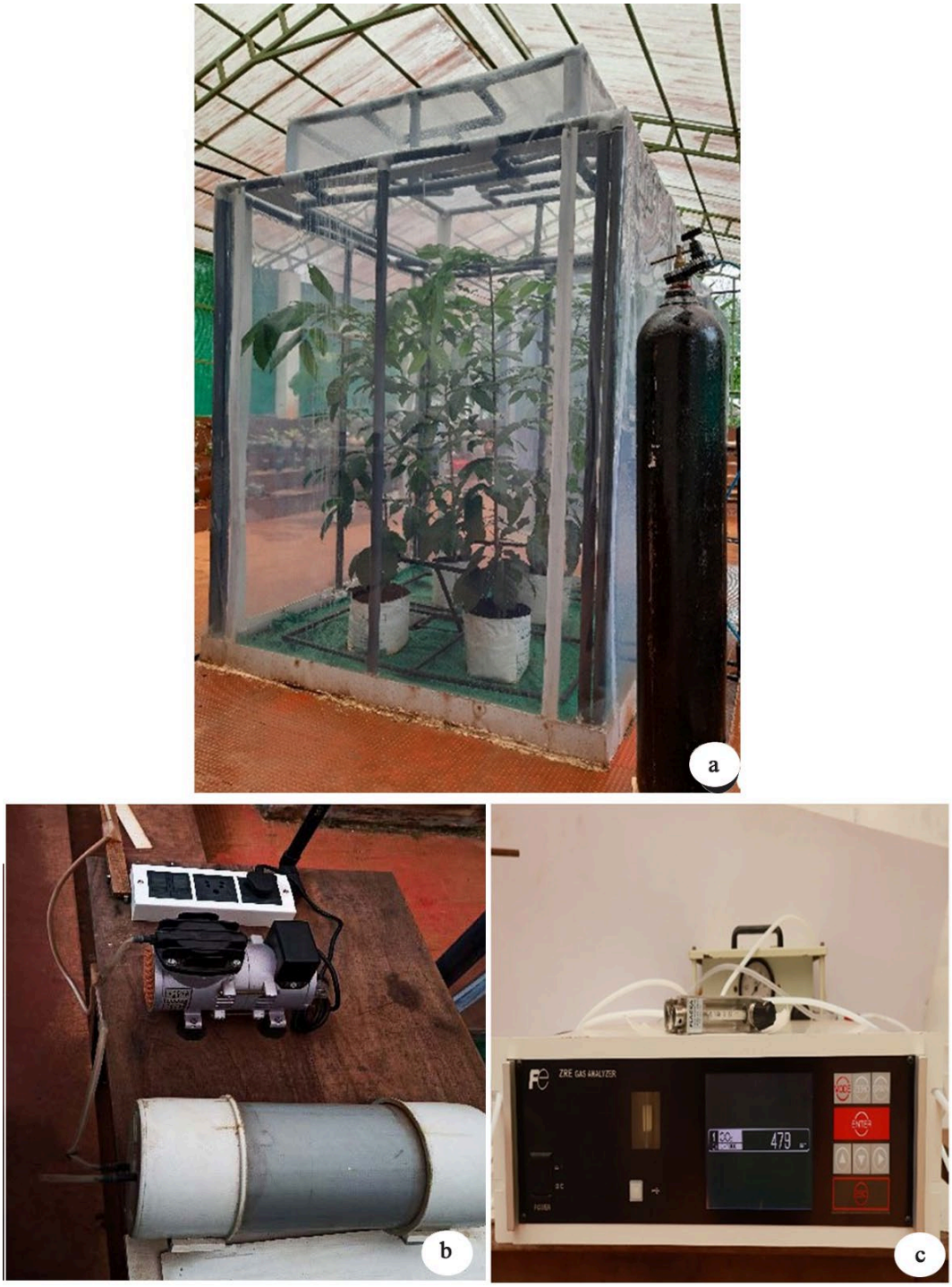


Plate 1.3.1: Facilities associated with Carbon dioxide-controlled growth chamber

a CO_2 Controlled experimental chamber, **b** Compressor and CO_2-air mixing tube,

c CO_2 analyzer

1.3.2 Standardization studies of growth chambers

The standardization study was undertaken to assess the retention and dissipation rate and thereby the daily flux of gases associated with both chambers. For this, the experimentation was undertaken in empty chambers (without plants). Before standardization studies, the efficiency of exhaust facilities, irrigation facilities, and CO_2 supply facilities associated with both chambers was monitored and ensured after several trials. The experimentation started at 9.00 am. The initial temperature and humidity inside both chambers were recorded using a thermometer/hygrometer, and the CO_2 using a CO_2 analyzer. Then the Treatment Chamber (TC) was supplied with a CO_2-air mixture, maintaining a resultant CO_2 concentration in the range of 900 to 1000 ppm. This was attained within a time of 15 minutes. Correspondingly, in the Control Chamber (CC), ambient air was supplied for 15 minutes. The temperature and humidity associated with the chambers were recorded along the the extent of CO_2. In the evening (6 p.m.), the temperature, humidity, and CO_2, inside both the CC and TC were recorded. The day flux of CO_2 was calculated by subtracting the amount of CO_2 retained in the chamber in the evening from the amount of CO_2 supplied in the morning. After measurements, the control and CO_2 treatment chambers were again supplied with air, and the air-CO_2 mixture, maintaining a CO_2 concentration in the range of 602 to 694 ppm in CC and 900 to 1000 ppm in the TC. The experiment is repeated the next day morning (9 a.m.). Night flux was calculated by subtracting the amount of CO_2 retained in the chamber the next morning from the amount of CO_2 supplied in the previous day's evening. The experiment was continued for 15 days.

The data concerning CO_2 (**Table 1.4.1 to 1.4.7**) and other microclimatic conditions (**Table 1.4.8 to 1.4.15**) within the chambers (CC, and TC) were used to validate the retention percentage and daily flux of CO_2 associated with the chambers in the absence of plants. The outcomes of the standardization studies were used for the validation of data obtained during experimentation with the respective tree species. The experimental setup for the Standardization study is shown in **Figures 1.3.2 and 1.3.3**

Figure 1.3.2: Standardization studies (control chamber)

Figure 1.3.3: Standardization studies (CO_2 treated chamber)

Identification/selection of plants and their multiplication and maintenance in nurseries for laboratory trials

After an extensive literature survey, six tree species, belonging to varying families were selected for the present study. The selection was based on their woody nature, duration of growth, adaptation to the existing environmental conditions, high biomass production, and expected carbon sequestration potentials. The taxonomic and botanical characteristics of the plants selected for the present study, along with their multiplication methods and nursery trials are detailed below. Selected plants were authenticated with the Division of Taxonomy, Department of Botany, University of Calicut. Voucher specimens were deposited at the Herbarium of the Department of Botany, University of Calicut (CALI).

***1) Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.** (CALI No. 7151)

Family: Combretaceae

Genus: *Terminalia*

Species: *arjuna*

Terminalia arjuna, a deciduous tree, is particularly prevalent in the sub-Himalayan regions and Eastern India (**Plate 1.3.2**). Its distribution is extensive throughout India and Sri Lanka. In Kerala, it is distributed in the Chinnar, Kulathupuzha, Pooyamkutty, and Tholpetty regions and is referred to as Neermaruthu or the Arjun tree. It is primarily cultivated along the banks of streams and rivers. The maximum

height of the tree is between 18.28 and 24.38 meters. The branches descend downwards from a vast, spreading crown that is adorned with a buttressed trunk. The leaves are simple and alternate in direction. The tree bears fleshy fruit, and its flowers are yellow. The tree undergoes flowering and fruiting from November to June (http://www.eflorakerala.com/plant_search.php). The trees are widely used in the wood industry, for the construction of houses, boats, and agricultural implements.. In Ayurvedic medicine, the desiccated bark is utilized extensively as a cardiotonic to treat obesity, blood disorders, urinary disorders, ulcers, and wounds. It grows rapidly and can flourish well on marginal and degraded lands producing higher biomass. Hence, their application in agroforestry and social forestry is extensive (Mokat *et al*., 2012). Multiplication is mainly through seeds. Early in the summer, seeds of *Terminalia arjuna* are sown in nursery beds. The germination of seeds begins within 8-12 days and is finished within 7-8 weeks. Drought stress levels in seedlings are greatest during germination and early growth phases (Kumar *et al*., 2010).

2) *Swietenia macrophylla* King (CALI No. 7155)

Family: Meliaceae

Genus: *Swietenia*

Species: *macrophylla*

Swietenia macrophylla is a deciduous and semi-evergreen tree of medium size, attaining a maximum height of 30–35 meters (**Plate 1.3.2**). It is native to subtropical and tropical regions of the world. It exhibits native distribution across the tropical regions of the Americas, including Mexico and Bolivia, in South America. The tree is cultivated in certain regions of North India. In Kerala, *Swietenia* is cultivated in plantations and traded at timber markets. The leaves are alternate and paripinnate, and the rachis is thin and glabrous. The fruit is desiccated, while the flower is bisexual and yellow. Seeds with wings are present. April to March is the season of blooming and bearing fruit (http://www.eflorakerala.com/plant_search.php). When young, the bark of the tree is flat and greyish. The wood is utilized for the construction of ships and boats. In addition to its conventional applications in hotels and public buildings, wood is also employed in the interior finishing of railway cars,

cases for delicate instruments such as scales, microscopes, microtones, and astronomical and surveying instruments (Larekeng *et al*., 2019).

3) *Pongamia pinnata* (L.) Pierre (CALI No. 7153)

Family: Fabaceae
Genus: *Pongamia*
Species: *pinnata*

Pongamia pinnata is native to Southeast Asia and the Indian subcontinent. This medium-sized tree has been successfully introduced to humid tropical regions, including portions of Australia, New Zealand, China, and the United States. It is an ornamental plant found throughout coastal India (**Plate 1.3.2**), inhabiting mangroves and deciduous forests It is also cultivated as an avenue tree. *P. pinnata* is frequently referred to as Ungu or Indian beach. The tree is also known by the local names Pongam, Ponnam, and Minnari. The species is distributed in the Kerala regions of Chinnar, Meenmutty, Nedumkayam, Peechi, Thenmala, and Walayar.

The leaves are imparipinnate and alternate. The tree bears dry fruit, and the flowers are white. The pod is a thick, flat substance with pointed ends. The colour of the bark on the tree is grey. The tree produces fruits and flowers between April and December. (http://www.eflorakerala.com). Throughout history, this plant has been used as a source of timber, fuel, animal fodder, dye, green manure, and for the production of strings and ropes in India and in neighboring regions (Reddy *et al*., 2015). This tree is extensively utilized in various afforestation practices and carbon mitigation initiatives (Scott *et al*., 2008; Bohre *et al*., 2014). Rooting was noted to be greater in cuttings derived from juvenile plants compared to mature trees. Additionally, seeds may be utilized to propagate *Pongamia pinnata* (Mukta and Sreevalli, 2010).

4) *Simarouba glauca* DC. (CALI No. 7152)

Family: Simaroubaceae
Genus: *Simarouba*
Species: *glauca*

One of the key avenue tree species in India for afforestation programs is *Simarouba glauca*, which can also help mitigate climate change. (**Plate 1.3.2**). It is an exotic

plant and is also used medicinally. The term "covered with a bloom," as denoted in the name *S. glauca*, pertains to the bluish-green foliage. It is a native of the Americas. Plantations of *S. glauca* can be found in the Indian states of Tamil Nadu, Orissa, Andhra Pradesh, and Karnataka. It exhibits a tropical distribution. The common names are the paradise tree, Lakshmitharu, etc. The tree's foliage consists of alternate, pinnate leaves. The fruit possesses a fleshy texture. The flower has a white and creamy colour. January to May are the months of blooming and bearing fruit (http://www.eflorakerala.com/plant_search.php). The evergreen tree maintains a robust root system that aids in groundwater conservation, microbial support, and prevention of soil erosion. The plant grows as an understory tree and is tolerant to shade. *S. glauca* is highly adaptable and possesses a rapid capacity for biomass production. Insect resistance notwithstanding, wood is frequently employed in the fabrication of furniture, toys, and pulp. It is lightweight, soft, possesses a low density, and is manageable. These factors guarantee that the local wood industries have a sufficient supply. Utilized products of the tree's wood include plywood cores and wood chips. A remedy for dysentery is the bark of the tree (Manasi and Gaikwad, 2011).

Seed germination normally ranges between 70 and 80 percent. The germination process commences on the fifteenth day after sowing and takes twenty-five days. In addition to eliminating the endocarp, pre-soaking seeds in cold water for 24 hours will enhance their germination. Pretreatment of the seed is superfluous due to its lack of dormancy. On the contrary, seeds may germinate more effectively if submerged in water for 12 hours before sowing (Sharma and Dwivedi, 2016).

5) *Mimusops elengi* L. (CALI No. 7156)
Family: Sapotaceae
Genus: *Mimusops*
Species: *elengi*

Mimusops elengi, an evergreen tree with a dense, rounded, and spreading crown, is commonly referred to as the Spanish cherry (**Plate 1.3.2**). Its natural habitat consists of semi-evergreen forests. The tree is also referred to as Bullet Wood, Elangi, Mukura, Bakulam, and West India Medlar. The average height is 15 to 30 meters; in exceptional cases, it can reach 40 meters. As an ornamental, it is frequently

cultivated in the tropics and subtropics to provide shade along roads and in gardens; and its fragrant flowers are highly valued. Baliga *et al*. (2011) describe the tree's indigenous range as the Western Ghat region of peninsular India. The leaves are spiral, simple, and alternate. The tree bears a fleshy fruit in the form of a berry. Its floral appearance is bisexual and white. December through August are the flowering months (http://www.eflorakerala.com/plant_search.php). Valued for its fragrant flowers, this ornamental plant is cultivated in the tropics and subtropics to provide shade along roads and in gardens. The tree is indigenous to the Western Ghats peninsula of India (Baliga *et al*., 2011). The leaves are spiral, simple, and alternate. The tree bears a fleshy fruit in the form of a berry. Its floral appearance is bisexual and white. December through August are the flowering months (http://www.eflorakerala.com/plant_search.php).

Commercial trade of wood is prevalent in certain tropical Asian nations. Heavy general construction, building purposes, boat and shipbuilding, agricultural implements, railway sleepers, and bridge construction are the areas utilizing wood. The timber is highly valuable. *Mimusops* fruit is also utilized in traditional medicine. The tree's thick bark has the appearance of being grayish-black or dark brownish-black. *Mimusops* fruits, seeds, and bark are utilized to treat dental conditions such as bleeding gums.

Propagation methods for *Mimusops elengi* include seed and cuttings. The germination rate is 70–90 percent and seeds produce offspring in 17–82 days (Gami *et al*., 2010).

6) *Syzygium cumini* (L.) Skeels (CALI No. 7154)

Family: Myrtaceae
Genus: *Syzygium*
Species: *cumini*

Syzygium cumini is a tropical evergreen tree that is highly valued for its ornamental value, fruit, and timber (**Plate 1.3.2**). Due to its rapid growth, this species can attain heights of up to 30 meters. It is frequently referred to as Njaval, black plum, or Java plum. Geographically, the tree's native range spans southern Asia, extending from

Africa and Madagascar. The tree is cultivated on the subcontinent of India. Chinnar, Choodal, Dhoni, Karimala, Karimutty, Kunthipuzha, the Nadukani ghats, Nedumkayam, Parambikulam, Ponmudi, Sholayar, Thekkady, Thrissur, Thirunelli, and Vallakkadavu are among the localities where it is distributed. The leaf is straight, opposite, and basally acute. As an aromatic myrtacean, its leaves are fragrant. Flowering occurs between December and April. Antibacterial leaves are utilized in the treatment of gums and teeth. Diabetes can be cured with seed powder (http://www.eflorakerala.com/plant_search.php). Due to its water resistance, the tree's wood is utilized to construct railway sleepers. According to Ayyanar and Subash Babu (2012), sore throats, asthma, and bronchitis can be alleviated with the tree's bark.

Propagation of *Syzygium cuumini* occurs via seeds and vegetative means. As a result of polyembryony, the progeny becomes viable via seed. Despite the increasing success of vegetative methods, seed propagation remains the preferred approach. *Syzygium* undergoes germination within a time frame of 10 to 15 days (Sanjay and Singh, 2006).

Production of saplings and their maintenance till the desired stage of growth

Seeds of *T. arjuna*, *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi*, and *S. cumini* were collected from the Kerala Forest Research Institute (KFRI), Peechi, Thrissur. For pre-treatment, the seeds were immersed in water for 24 hours. However, for *Swietenia macrophylla,* as the seeds have a tough outer coating, they were subjected to a 30-minute soaking in hot water followed by scrubbing with sandpaper.

For rearing plantlets, grow bags of dimension 35*20*20 cm were filled with soil, sand, and cow dung in a ratio of 3:1:1. The seeds were sown and watered regularly. After germination, the healthy plantlets of *T. arjuna, S. macrophylla, P. pinnata, S. glauca, S. cumini,* and *M. elengi* were transferred to other sets of grow bags and watered regularly. They were then retained in the polyhouse for acclimatization for about 18 months. The uniformly grown healthy plantlets (12 each) were subsequently taken for experimentation.

Plate 1.3.2: Plants selected for experimentation. a) *Terminalia arjuna* b) *Swietenia macrophylla*, c) *Pongamia pinnata* d) *Simarouba glauca* e) *Mimusops elengi* f) *Syzygium cumini*

1.3.3 Monitoring the responses of individual tree species to elevated CO_2 and assessment of microclimatic conditions.

Eighteen-month-old plantlets of *T. arjuna*, *S. macrophylla*, *P. pinnata, S. glauca, M. elengi,* and *S. cumini* were selected separately for the experimentation. For each study, 2 sets of plantlets were taken, in which one set was retained in the CC, and the other in the TC. Both the chambers were sealed from the outside to prevent the exchange of air. As per the methodology outlined for the standardization study, for about 15 minutes in the morning (9 a.m.), ambient air was pumped into the CC, ensuring a consistent ambient CO_2 concentration. Similarly, the CO_2-air mixture was introduced into the TC for approximately 15 minutes in the morning (9 a.m.), ensuring an elevated CO_2 concentration. The monitoring of CO_2 concentration in the CC as well as the TC was accomplished through an automated CO_2 analyzer (Fuji Electric NDIR type Infrared Gas Analyzer). Along with the CO_2 concentration (ppm), temperature (^oC), and humidity (%) within the chambers were also monitored using a Billion Bag digital wireless electronic hygrothermometer.

The experiment is repeated at 6:00 p.m., with the monitoring of CO_2, temperature (^oC), and humidity (%) before and after CO_2 supplementation. Accordingly, the day flux of CO_2 was calculated by subtracting the amount of CO_2 retained in the chamber in the evening from that of the amount of CO_2 supplied in the morning. The experiment was repeated the next day morning (9 a.m.) and the night flux was calculated by subtracting the amount of CO_2 retained in the chamber the next morning from the amount of CO_2 retained in the evening of the previous day. The experiment was continued for 15 days, as outlined in the standardization studies. Meantime the changes in the growth and biochemical attributes associated with the plants under experimentation were monitored and are dealt with in Chapter II. The experimentation using chambers (both control and CO_2 treated) with *Terminalia arjuna* (**Figure 1.3.4 a,b**), *Swietenia macrophylla* (**Figure 1.3.4 c,d**), *Pongamia pinnata* **(Figure 1.3.4 e,f)** *Simarouba glauca* (**Figure 1.3.4 g,h**)*, Mimusops elengi* (**Figure 1.3.4 i, j)** and *Syzygium cumini* **(Figure 1.3.4 k,l)** are depicted.

Figure 1.3.4a Studies with *T. arjuna* (Control Chamber)

Figure 1.3.4b Studies with *T. arjuna* (CO_2 treated Chamber).

Figure 1.3.4c Studies with *S. macrophylla* (Control Chamber)

Figure 1.3.4d Studies with *S. macrophylla* (CO_2 treated chamber)

Figure 1.3.4e Studies with *P. pinnata* (Control Chamber)

Figure 1.3.4f Studies with *P. pinnata* (CO_2 treated Chamber)

Figure 1.3.4g Studies with *S. glauca* (Control Chamber)

Figure 1.3.4h Studies with *S. glauca* (CO_2 treated Chamber)

Figure 1.3.4i Studies with *M. elengi* (Control Chamber)

Figure 1.3.4j Studies with *M. elengi* (CO_2 treated Chamber)

Figure 1.3.4k Studies with *S. cumini* (Control Chamber)

Figure 1.3.4l Studies with *S. cumini* (CO_2 treated Chamber)

1.3.4 Statistical analysis

All statistical tests were done using R statistical software. Shapiro-Wilk normality test was used to test the normality of data in the day flux of CO_2 between control and treatment. Non-parametric Wilcoxon signed-rank test was performed to test if there is a significant difference between control and treatment in the day flux of CO_2. The same test was used to test if there was any significant difference between

the control and treatment in day temperature and night temperature. Kruskal Wallis rank sum test was performed to test if there is a significant difference in the day flux of CO_2 between the plants under elevated CO_2 conditions, followed by a pairwise Wilcoxon test between all possible pairs of groups with Bonferroni adjusted p values.

1.4 RESULTS

The results of the present study are represented in two sessions: Session I, which deals with the standardization of the empty growth chambers (without plants), concerning the extent of CO_2 and other microclimatic conditions consequent to the supply of air (CC) and air-CO_2 mixture (TC), and Session II deals with the changes in the CO_2 and other microclimatic conditions associated with the chambers supplied with air (CC) and air- CO_2 mixture (TC) attributed by the growth of the saplings of six tree species.,

In both Standardisation studies and studies using the plant species, the day flux in CC and TC is calculated from the amount of CO_2 supplied in the morning with that of the CO_2 retained in the evening. From these results, the percentage of day flux is calculated. Similarly, the night flux (CC and TC) in both standardization studies and studies using the plant species is calculated by subtracting the amount of CO_2 in the next day's morning from the CO_2 supplied in the previous day's evening. The percentage of night flux is calculated from the individual values of night flux. The negative value of CO_2 flux in the chamber indicates a reduction of CO_2 in the chamber and a positive value indicates the attribution of CO_2 to the chamber.

Session I (CO_2**)**

1.4.1 Carbon dioxide flux associated with standardization studies

In the treated chamber, CO_2 after supply ranged from 996 to 1030 ppm. Day flux of CO_2 is estimated as the difference in the extent of CO_2 supplied in the morning with that of the CO_2 retained in the evening. Similarly, night flux is assessed as the extent of CO_2 in the evening with that of its extent in the next morning. The day and night flux of CO_2 associated with the control and the CO_2-treated chambers are depicted in **Table 1.4.1**. In the CC average value of CO_2 before and after the air supply is calculated from the experimentation of 15 days. The estimate of CO_2 before air supply is 664.5±26.99 ppm and after supply is 665±23.26 ppm. The average value of

CO_2 retained in the chamber in the evening is 634.2±11.56 ppm. The average value of CO_2 in the next day morning is 671.7±13.7ppm. The day flux maintained in the chamber is -30.71±17.94. The percentage change in day flux is -4.543±2.662. In the treated chamber, the average level of CO_2 supplied is 1018.4 ppm. The average value of CO_2 in the evening is 941.3 ppm. In the treated chamber, the day flux of CO_2 is noted to be -77.07 ppm, and the night flux is 24.71 ppm.

Session II (CO_2)

1.4.2 Carbon dioxide flux associated with the plants under experimentation

The levels of CO_2 after supply range from 900 to 1090 ppm in the CO_2-treated chamber having *T. arjuna*. The average day flux retained in the CC with *T. arjuna* is -2.857±21.83 and the night flux is 17.93±38.33. Similarly, the average day flux in the TC with *T. arjuna* is -473.4±47.14 ppm and the night flux is 12.36±65.80 **(Table 1.4.2).** Day flux in the TC is higher than in the CC which indicates more assimilation of CO_2 by the plant in the CO_2 treated chamber.

The CO_2 after supply ranges from 990 to 1030 ppm in the TC with *S. macrophylla*. The average day flux maintained in the CC with *S. macrophylla* is 1.071±35.88 and the night flux is 5.5±32.76. The average day flux in the TC with *S. macrophylla* is -517.5±16.84 and the night flux is -0.071±34.66 as depicted in **table 1.4.3**. Here also the day flux is higher than the night flux.

The CO_2 after supply ranges from 1007 to 1090 ppm in TC with *P. pinnata.* The average day flux retained in the CC with *P. pinnata* is -4.5 ±22.73 and the night flux is 22.07±26.62. The average day flux retained in the TC is -467.50±37.86 and the night flux is 104.8±91.52 **(Table 1.4.4).**

The CO_2 measure ranges from 860 to 1100 ppm in the CO_2 flux studies with *S. glauca.* The average day flux retained in the CC with *S. glauca* is -179.6±115.5 and the night flux is 184.4± 86.27. The average day flux retained in the TC with *S. glauca* is -425.2±131.8 and the night flux is 406.6±128.8 as shown in **table 1.4.5**.

The CO_2 levels after supply range from 910 to 1020 ppm in *M. elengi*. The average day flux retained in the CC with *M. elengi* is -0.857±27.73 and the night flux is 11.21±26.49. The average day flux retained in the treated chamber with *M. elengi* is -258.8±95.36 and the night flux is -0.643±79.90 **(Table 1.4.6).**

In the case of *S. cumini* the levels of CO_2 after supply ranges from 840 to 1025 ppm. The average day flux retained in the CC with *S. cumini* is -32.57±42.23 and the night flux is 33.43± 28.59. The average day flux retained in the treated chamber with *S. cumini* is -265.9± 73.95 and the night flux is 57.71±29.75 which is represented in **table 1.4.7.**

The actual day and night flux of CO_2 for each plant inside the CC and TC is calculated by subtracting the day and night flux concerning the standardization study from the given value. In *T. arjuna* a reduction of -77.07 ppm of CO_2 from the mean value of -473.4 ppm of day flux is noticed in the treated chamber which indicates that the day flux is -396.33 ppm indicating the CO_2 assimilation efficiency of *T arjuna* in the day time. In *S. macrophylla* the day flux inside the TC is obtained by subtracting the day flux of the Standardization study inside the treated chamber from the day flux of the plant. Thus *S. macrophylla* has a day flux value of -440.43ppm CO_2. Similarly, *P. pinnata*, *S.glauca*, *M. elengi,* and *S. cumini* have a day flux of -390.43 ppm, 348.13 ppm, -181.73 ppm, and -188.83 ppm respectively.

The average day flux of *T. arjuna*, *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi*, *S. cumini* inside the CC after subtracting the day flux of Standardisation study is -25.85 ppm, 31.78 ppm, -26.21 ppm, -148.89 ppm, 29.85 ppm, -1.83 ppm respectively. The average night flux inside the CC with *T. arjuna* , *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi*, *S. cumini* is found to be -19.5ppm, -31.93 ppm, -15.36 ppm, -146.97 ppm, -26.22 ppm, -4 ppm after subtracting the value of night flux in the standardization study. The average night flux inside the TC with *T. arjuna*, *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi*, *S. cumini* is found to be -12.35 ppm, -24.78 ppm, 80.09 ppm, 381.89 ppm, -25.35 ppm, 33 ppm after subtracting the value of night flux in the Standardisation study.

When the percentage change in day flux of each plant inside the TC is compared, it is observed that *S. macrophylla* has the highest sequestration efficiency, followed by *T. arjuna*, *P. pinnata*, *S. glauca*, *S. cumini*, *M. elengi*. The percentage change in day flux inside the treated chamber for these plants were -51.68, -47.60, -44.32, -39.48, -28.99, and -27.29, respectively. Percentage change in day flux in CCs with *T. arjuna*, *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi* and *S. cumini* is -0.383, 0.713, -0.775, -23.78, -0.019, -5.064. The percentage change in night flux inside CC

and TC with *T. arjuna is* 3.416 and 2.296 respectively. The percentage change in night flux inside CC and TC with *S. macrohylla* is 1.495 and 0.063. Similarly, *P. pinnata* (control - 4.454%) (treated -18.15%). *S. glauca* (control - 35.3%) (treated - 66.48%). *M. elengi* (control- 2.236%) (treated- 00.501) is also represented. In *S. cumini* percentage change in night flux in CC and TC respectively is 6.007% and 8.936%. This is the first study to estimate CO_2 reduction in ppm (plant uptake) values.

The non-parametric Wilcoxon signed-rank test was used to test whether there was any significant difference in the CO_2 flux between control and treatment samples of all the plants except *S. glauca,* as the data did not meet the normality assumption. A significant difference between control and treated is observed in *T. arjuna* ($p < 0.001$), *S. macrophylla* ($p < 0.001$), *P. pinnata* ($p < 0.001$), *S. glauca* ($p < 0.001$), *M. elengi* ($p < 0.001$), and *S. cumini* ($p < 0.001$) **(Figure 1.4.1, Annexure 1)**. Kruskal-Wallis rank sum test reported significant differences in the day flux of CO_2 between the plants under elevated CO_2 conditions ($H = 52.619$, $df = 5$, $p < 0.05$). The Pairwise Wilcoxon test was carried out on all possible pairs of groups after Bonferroni adjustment. The descriptive statistics (below) give the median values for the flux of control and treatment, considering the confidence interval to be 95 %. In *T.* arjuna, median values for the flux of control and treatment groups are 1.5 and -488.5. In *S. macrophylla*, the median values for the flux of control and treatment groups are -2.5 and -515. In *P. pinnata* it is -2.5 and -473.5 respectively. In *M. elengi*, the median values for the flux of control and treatment are 7.5 and -257.5. In *S. cumini* the values are -13.5 and -264.5 respectively. The green line in the figure represents control and the red line represents treated. A higher negative value of day flux inside the treated chamber compared to control for all the plants indicates a reduction of CO_2 in the chamber due to the consumption of CO_2 by the plants. It is evident from the figure that *S. macrophylla* consumes more CO_2 under elevated CO_2 conditions compared to other plants.

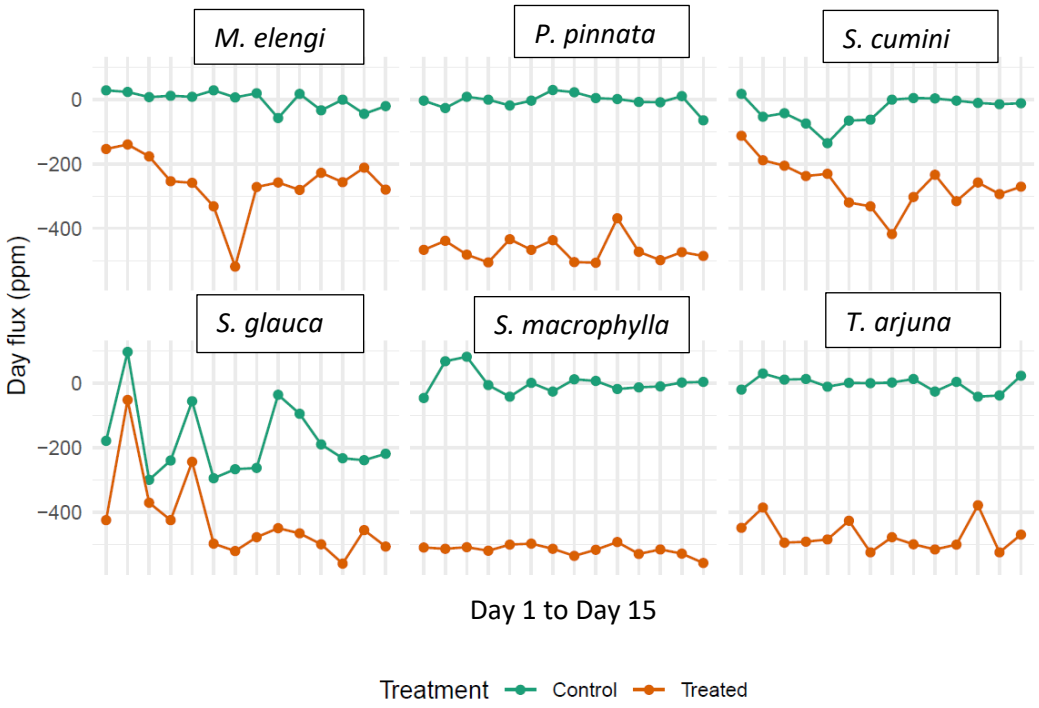


Figure 1.4.1: Variation of day flux of CO_2 in control and treated sets

Table 1.4.1: Standardization studies on experimental chambers (CO_2 flux)

Control chamber										CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change		Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	583	602	617	656	15	2.492	39	6.321		615	1025	955	973	-70	-6.829	18	1.885
2	656	660	622	680	-38	-5.758	58	9.325		973	996	938	970	-58	-5.823	61	6.503
3	680	685	622	649	-63	-9.197	27	4.341		970	999	942	978	-57	-5.706	36	3.822
4	649	650	622	660	-28	-4.308	38	6.109		978	1020	947	949	-73	-7.157	2	0.211
5	660	653	627	649	-26	-3.982	22	3.509		949	1026	941	960	-85	-8.285	19	2.019
6	649	647	625	671	-22	-3.400	46	7.360		960	1005	929	940	-76	-7.562	11	1.184
7	671	658	641	670	-17	-2.584	29	4.524		940	1030	949	962	-81	-7.864	13	1.370
8	670	670	637	676	-33	-4.925	39	6.122		962	1001	945	973	-56	-5.594	28	2.963
9	676	671	639	687	-32	-4.769	48	7.512		973	1025	929	959	-96	-9.366	30	3.229
10	687	687	646	687	-41	-5.968	41	6.347		959	1045	936	955	-109	-10.43	19	2.030
11	687	680	644	672	-36	-5.294	28	4.348		955	1009	941	963	-68	-6.739	22	2.338
12	672	673	654	674	-19	-2.823	20	3.058		963	1030	936	953	-94	-9.126	17	1.816
13	674	680	638	690	-42	-6.176	52	8.150		953	1025	930	976	-95	-9.268	46	4.946
14	690	694	646	683	-48	-6.916	37	5.728		976	1022	961	985	-61	-5.969	24	2.497
Avg	664.5	665.00	634.2	671.7	-30.71	-4.543	37.43	5.911		937.5	1018.4	941.3	964.00	-77.07	-7.551	24.71	2.629
Sd	26.99	23.26	11.56	13.70	17.94	2.662	11.26	1.809		93.48	14.22	9.52	12.49	16.83	1.561	15.08	1.613

Table 1.4.2: CO_2 flux studies on *Terminalia arjuna* using experimental chambers

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	496	478	458	463	-20	-4.184	5	1.080	475	945	496	483	-449	-47.51	-13	-2.621
2	463	446	476	445	30	6.726	-30	-6.742	483	900	514	474	-386	-42.88	-40	-7.782
3	445	431	456	442	11	2.552	-14	-3.167	474	1000	505	472	-495	-49.50	-33	-6.535
4	442	432	445	476	13	3.009	31	6.513	472	1009	517	477	-492	-48.76	-40	-7.737
5	476	457	446	475	-11	-2.407	29	6.105	477	988	503	543	-485	-49.08	40	7.952
6	475	463	464	464	1	0.216	0	0.000	543	1012	585	577	-427	-42.19	-8	-1.368
7	464	446	446	444	0	0.000	-2	-0.450	577	1024	499	487	-525	-51.27	-12	-2.405
8	444	431	433	440	2	0.464	7	1.591	487	990	510	479	-478	-48.28	-33	-6.471
9	440	420	433	475	13	3.095	42	8.842	479	970	470	572	-500	-51.54	102	21.70
10	475	445	419	447	-26	-5.843	28	6.264	572	1002	486	602	-516	-51.49	116	23.86
11	447	442	446	574	4	0.905	128	22.30	602	1090	589	744	-501	-45.96	155	26.31
12	574	566	424	447	-42	-7.420	23	5.145	744	1012	633	626	-379	-37.45	-7	-1.106
13	447	445	407	385	-38	-8.539	-22	-5.714	626	990	465	409	-525	-53.03	-56	-12.04
14	385	380	403	429	23	6.053	26	6.061	409	990	520	522	-470	-47.47	2	0.385
Avg	462.36	448.71	439.7	457.5	-2.857	-0.383	17.93	3.416	530.00	994.4	520.8	533.3	-473.4	-47.60	12.36	2.296
Sd	41.24	40.60	21.19	40.93	21.83	4.734	38.33	7.265	86.81	42.05	48.12	85.42	47.14	4.28	65.80	12.67

Table 1.4.3: CO_2 flux studies on *Swietenia macrophylla* using experimental chambers

Control chamber										CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change		Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	542	516	562	411	-46	-8.915	-59	-10.49		552	990	480	438	-510	-51.51	-42	-8.750
2	411	409	477	405	68	16.62	-72	-15.09		438	1008	494	433	-514	-50.99	-61	-12.34
3	405	400	482	482	82	20.50	0	0.000		433	998	489	475	-509	-51.01	-14	-2.863
4	482	462	456	493	-6	-1.299	37	8.114		475	998	478	535	-520	-52.10	57	11.92
5	493	497	455	472	-42	-8.451	17	3.736		535	990	489	486	-501	-50.61	-3	-0.613
6	472	459	460	494	1	0.218	34	7.391		486	990	492	500	-498	-50.30	8	1.626
7	494	475	449	449	-26	-5.474	21	4.677		500	990	476	470	-514	-51.91	-6	-1.261
8	449	433	445	466	12	2.771	21	4.719		470	1011	475	481	-536	-53.01	6	1.263
9	466	439	446	448	7	1.595	2	0.448		481	999	482	464	-517	-51.75	-18	-3.734
10	448	469	451	486	-18	-3.838	35	7.761		464	995	502	515	-493	-49.54	13	2.590
11	486	459	446	445	-13	-2.832	-1	-0.224		515	1030	500	460	-530	-51.45	-40	-8.000
12	445	431	421	446	-10	-2.320	25	5.938		460	995	479	525	-516	-51.85	46	9.603
13	446	429	431	437	2	0.466	6	1.392		525	1002	473	478	-529	-52.79	5	1.057
14	437	425	429	440	4	0.941	11	2.564		478	1020	462	510	-558	-54.70	48	10.39
Avg	462.57	450.21	457.86	455.29	1.071	0.713	5.50	1.495		486.5	1001.1	483.6	483.5	-517.5	-51.68	-0.071	0.063
Sd	36.01	32.67	34.30	28.17	35.88	8.373	32.76	6.734		35.14	12.18	11.17	30.62	16.84	1.27	34.66	7.187

Table 1.4.4: CO_2 flux studies on *Pongamia pinnata* using experimental chambers

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	487	488	484	530	-4	-0.820	46	9.504	489	1057	590	850	-467	-44.18	260	44.06
2	530	516	489	496	-27	-5.233	7	1.431	850	1044	605	646	-439	-42.05	41	6.777
3	496	490	498	506	8	1.633	8	1.606	646	1075	593	647	-482	-44.83	54	9.106
4	506	494	493	490	-1	-0.202	-3	-0.609	647	1080	574	620	-506	-46.85	46	8.014
5	490	518	499	521	-19	-3.668	22	4.409	620	1015	581	646	-434	-42.75	65	11.18
6	521	506	502	498	-4	-0.791	-4	-0.797	646	1050	583	573	-467	-44.47	-10	-1.715
7	498	493	522	495	29	5.882	-27	-5.172	573	1041	604	583	-437	-41.97	-21	-3.477
8	495	481	503	516	22	4.574	13	2.584	583	1048	543	673	-505	-48.18	130	23.94
9	516	503	507	538	4	0.795	31	6.114	673	1073	566	705	-507	-47.25	104	18.37
10	538	498	499	525	1	0.201	26	5.210	705	1007	638	737	-369	-36.64	67	10.50
11	525	504	496	536	-8	-1.587	40	8.065	737	1053	580	726	-473	-44.91	157	27.06
12	536	519	510	534	-9	-1.734	24	4.706	726	1090	591	754	-499	-45.78	135	22.84
13	534	515	525	570	10	1.942	45	8.571	754	1085	611	847	-474	-43.68	143	23.40
14	570	549	484	565	-65	-11.84	81	16.74	847	1036	550	847	-486	-46.91	297	54.00
Avg	517.2	505.2	500.7	522.8	-4.50	-0.775	22.07	4.454	678.2	1053.8	586.3	703.8	-467.50	-44.32	104.8	18.15
Sd	23.51	17.39	12.30	24.98	22.73	4.315	26.62	5.381	101.00	24.99	24.59	94.50	37.86	2.93	91.52	16.18

Table 1.4.5: CO_2 flux studies on *Simarouba glauca* using experimental chambers

Control chamber										CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change		Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	516	518	339	523	-179	-34.55	184	54.27		647	1047	622	1100	-425	-40.59	478	76.84
2	523	630	727	896	97	15.39	169	23.24		1100	1100	1048	1100	-52	-4.727	52	4.962
3	896	896	596	842	-300	-33.48	246	41.27		1100	1100	729	1100	-371	-33.72	371	50.89
4	842	842	602	636	-240	-28.50	34	5.648		1100	1100	675	1042	-425	-38.63	367	54.37
5	636	636	580	831	-56	-8.80	251	43.27		1042	860	616	1100	-244	-28.37	484	78.57
6	831	831	536	807	-295	-35.49	271	50.56		1100	1100	602	1090	-498	-45.27	488	81.06
7	807	807	540	809	-267	-33.08	269	49.81		1090	1100	579	1044	-521	-47.36	465	80.31
8	809	809	546	616	-263	-32.51	70	12.82		1044	1100	622	845	-478	-43.45	223	35.85
9	616	616	580	595	-36	-5.844	15	2.586		845	1100	650	1068	-450	-40.90	418	64.30
10	595	595	500	698	-95	-15.96	198	39.60		1068	1020	554	1020	-466	-45.68	466	84.11
11	698	698	508	737	-190	-27.22	229	45.07		1020	1100	600	1058	-500	-45.45	458	76.33
12	737	737	504	772	-233	-31.61	268	53.17		1058	1100	540	1100	-560	-50.90	560	103.7
13	772	772	533	724	-239	-30.95	191	35.83		1100	1100	644	1100	-456	-41.45	456	70.80
14	724	724	505	692	-219	-30.24	187	37.03		1100	1100	593	1000	-507	-46.091	407	68.63
Avg	714.4	722.2	542.5	727.00	-179.6	-23.78	184.4	35.30		1029.5	1073.3	648.1	1054.7	-425.2	-39.48	406.6	66.48
Sd	121.10	110.7	83.53	107.2	115.5	14.78	86.27	17.41		129.1	66.14	124.7	69.17	131.8	11.54	128.8	24.17

Table 1.4.6: CO_2 flux studies on *Mimusops elengi* using experimental chambers

Control chamber									CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	526	518	546	537	28	5.405	-9	-1.648	634	970	816	726	-154	-15.87	-90	-11.02
2	537	530	553	540	23	4.340	-13	-2.351	726	918	778	720	-140	-15.25	-58	-7.455
3	540	534	541	543	7	1.311	2	0.370	720	900	723	708	-177	-19.66	-15	-2.075
4	543	525	536	551	11	2.095	15	2.799	708	937	683	703	-254	-27.10	20	2.928
5	551	523	531	516	8	1.530	-15	-2.825	703	910	651	567	-259	-28.46	-84	-12.90
6	516	508	536	535	28	5.512	-1	-0.187	567	917	585	500	-332	-36.20	-85	-14.53
7	535	515	521	514	6	1.165	-7	-1.344	500	1019	500	594	-519	-50.93	94	18.80
8	514	506	525	584	19	3.755	59	11.238	594	913	641	734	-272	-29.79	93	14.50
9	584	568	510	510	-58	-10.21	0	0.000	734	1020	762	629	-258	-25.29	-133	-17.45
10	510	486	503	555	17	3.498	52	10.33	629	930	649	766	-281	-30.21	117	18.02
11	555	548	514	507	-34	-6.204	-7	-1.362	766	920	692	715	-228	-24.78	23	3.324
12	507	498	497	551	-1	-0.201	54	10.86	715	966	709	778	-257	-26.60	69	9.732
13	551	545	500	531	-45	-8.257	31	6.200	778	965	753	802	-212	-21.96	49	6.507
14	531	525	504	500	-21	-4.000	-4	-0.794	802	936	656	665	-280	-29.91	-9	-1.372
Avg	535.71	523.50	522.64	536.46	-0.857	-0.019	11.21	2.236	684.00	944.3	685.5	687.8	-258.8	-27.29	-0.643	0.501
Sd	20.95	21.31	18.35	21.61	27.73	5.125	26.49	5.175	86.87	38.57	82.22	89.17	95.36	8.92	79.903	12.11

Table 1.4.7: CO_2 flux studies on *Syzygium cumini* using experimental chambers

Control chamber										CO_2 treated chamber							
Days of experimentation	Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day morning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change		Estimate of CO_2 (ppm) before supply	Estimate of CO_2 (ppm) after supply	Estimate of CO_2 (ppm) in the evening	Estimate of CO_2 (ppm) in the next day mmorning	Day Flux (ppm)	Day Flux Percentage change	Night Flux (ppm)	Night Flux Percentage change
1	544	538	555	632	17	3.160	77	13.87		537	840	727	808	-113	-13.45	81	11.14
2	632	626	572	600	-54	-8.626	28	4.895		808	858	669	700	-189	-22.02	31	4.634
3	600	595	552	649	-43	-7.227	97	17.57		700	850	644	746	-206	-24.23	102	15.83
4	649	644	569	624	-75	-11.646	55	9.666		746	870	632	707	-238	-27.35	75	11.86
5	624	714	578	637	-136	-19.048	59	10.20		707	893	662	737	-231	-25.86	75	11.32
6	637	634	568	600	-66	-10.410	32	5.634		737	986	666	710	-320	-32.45	44	6.607
7	600	595	532	560	-63	-10.588	28	5.263		710	920	588	660	-332	-36.08	72	12.24
8	560	555	554	575	-1	-0.180	21	3.791		660	1025	607	606	-418	-40.78	-1	-0.165
9	575	534	538	536	4	0.749	-2	-0.372		606	885	582	594	-303	-34.23	12	2.062
10	536	533	536	543	3	0.563	7	1.306		594	908	674	725	-234	-25.77	51	7.567
11	543	540	536	563	-4	-0.741	27	5.037		725	940	624	723	-316	-33.61	99	15.86
12	563	552	541	560	-11	-1.993	19	3.512		723	908	650	707	-258	-28.41	57	8.769
13	560	553	538	553	-15	-2.712	15	2.788		707	930	636	691	-294	-31.61	55	8.648
14	553	546	534	539	-12	-2.198	5	0.936		691	903	632	687	-271	-30.01	55	8.703
Avg	584.00	582.79	550.2	583.6	-32.57	-5.064	33.43	6.007		689.3	908.2	642.3	700.0	-265.9	-28.99	57.71	8.936
Sd	38.90	54.36	16.03	39.32	42.23	6.288	28.59	5.110		69.75	51.24	37.54	54.37	73.95	6.76	29.75	4.656

Similarly, the results of the fluctuations in temperature associated with the chambers under standardization studies and studies utilizing plants are depicted below:

Session I

1.4.3 Temperature variations within the chambers associated with Standardization studies

In the CC, the temperature after air supplementation varies from 36.70 to 45°C whereas in the TC temperature varies from 37 to 44.2 °C after CO_2 supplementation. Evening temperature in the CC and TC vary in the range of 32.30 to 37.30 °C and 32.5 to 37.1°C respectively. The percentage change in day variation in temperature in CC and TC is calculated and found to be 13.31±5.818 and 13.89±5.109. The percentage change in night variation in temperature in CC and TC are found to be 13.52±7.478 and 15.51±6.913 respectively **(Table 1.4.8).**

Session II

1.4.4 Temperature variations within the chamber under plant growth

Wilcoxon test for the change in day and night temperature in CC and TC in all the plants is depicted in **Annexures 2 and 3** respectively.

In the CC with *T. arjuna* the temperature after air supplementation varies from 26 to 43°C whereas in the treated chamber, temperature varies from 27 to 45°C after CO_2 supplementation. Evening temperature in the CC and TC vary in the range of 29.5 to 40.5°C and 28.5 to 35.90°C respectively. The percentage change in day variation in temperature in CC and TC is calculated and found to be 15.05±9.43 and 15.95±8.96 respectively. The percentage change in night variation in temperature in CC and TC is 18.18±17.21 and 13.08±12.66 respectively **(Table 1.4.9).** The change in night variation in temperature in CC and TC was significant ($v = 85$, $p < 0.05$).

In the CC with *S. macrophylla,* there is only a slight change in the average temperature in the morning before and after air supplementation and before and after CO_2 supply. The increase in temperature after CO_2 supplementation is higher in the TC compared to that of the CC. The evening temperature in the CC and TCs is almost the same. The percentage change in day variation in temperature in CC and TC are found to be 17.92±12.43 and 15.10±12.40 respectively. The percentage change in night variation in temperature in CC and TC are found to be 23.26±13.03 and 16.96±10.99 respectively **(Table 1.4.10).** The change in day ($v=6$, $p < 0.05$) and

night variation in temperature in CC and TC between the initial and final day was significant (v=97.5, $p < 0.05$).

Percentage change in day variation in CC and TC with *P. pinnata* is 7.50±7.419 and 5.17±6.784 respectively while percentage change in night variation in CC and TC are found to be 1.04±9.292 and 0.54±6.11 respectively. The change in day temperature variation in CC and TC between the initial and final day was found to be significant (v=14, $p < 0.05$). The temperature in the evening in both chambers is found to be the same **(Table 1.4.11)**.

The temperature in the morning before and after the air supplementation in the CC with *S. glauca* varies from 28.6 to 34.7°C and 28.7 to 42.2°C. The temperature in the morning before and after CO_2 supplementation in the TC varies from 28.10 to 36.5°C and 28.5 to 41.7°C respectively. The temperature in the evening in the CC and the TC is 31.81±2.557 and 31.82±2.528. The percentage change in day variation in the CC and TC is 12.06±4.057 and 11.30±4.324 while the percentage change in night variation in the CC and TC is 1.67±9.766 and 0.93±9.189 respectively **(Table 1.4.12)**. The change in day temperature variation in CC and TC between the initial and final day was found to be significant (v =10, $p < 0.05$).

The temperature in the morning before and after the air supplementation in the CC with *M. elengi* varies from 28.6 to 41.3°C and 28.7 to 48.8°C. The temperature in the morning before and after CO_2 supplementation in the treated chamber varies from 28.80 to 40.90°C and 28.7 to 41.4°C. The temperature in the evening in both chambers remains the same. The percentage change in day variation in the CC and the TC is 11.78±10.14 and 7.78±8.47 while the percentage change in night variation in the CC and the TC is 10.64±12.26 and 7.68±14.01 respectively **(Table 1.4.13).** The change in day temperature variation in CC and TC between the initial and final day was found to be significant (v=8.5, $p < 0.05$). The change in night temperature variation in CC and TC between the initial and final day was found to be significant (v=99, $p < 0.05$).

The temperature in the morning before and after air supplementation in the CC with *S. cumini* varies from 29.6 to 40°C and 30.30 to 40.50°C respectively while in the TC temperature varies from 29 to 38.80°C before CO_2 supplementation and varies from 29.80 to 39.60°C after CO_2 supplementation. There is only a slight change in

the temperature in the evening in both chambers. Percentage change in the day variation in temperature in the CC and the TC is found to be 6.95±7.615 and 3.14±8.14 while percentage change in the night variation in temperature in both the CC and the TC is found to be 4.94±10.75 and 1.10±9.99 which is represented in **table 1.4.14.** The change in temperature day variation in CC and TC between the initial and final day was significant ($v=0$, $p < 0.05$). The change in temperature night variation in CC and TC between the initial and final day was found to be significant ($v=105$, $p < 0.05$). **Figure 1.4.2** represents the change in day flux of CO_2 with day temperature and **figure 1.4.3** represents the change in night flux of CO_2 with night temperature. In this figure, the solid line represents the change in day and night temperature in CC and TC. The dotted line represents the change in day and night flux of CO_2 between CC and TC. The CC is indicated by a red line and TC is represented by a green line.

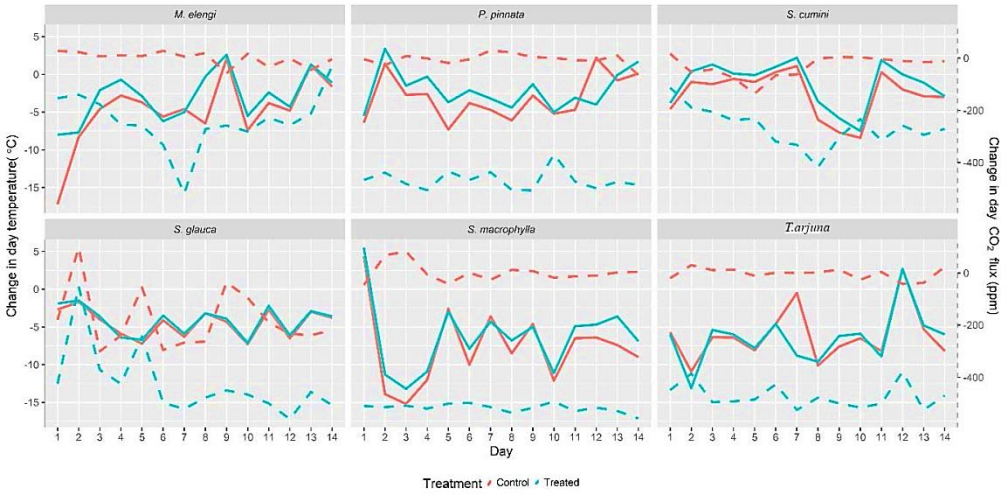


Figure 1.4.2: Changes in day flux of CO_2 with day temperature

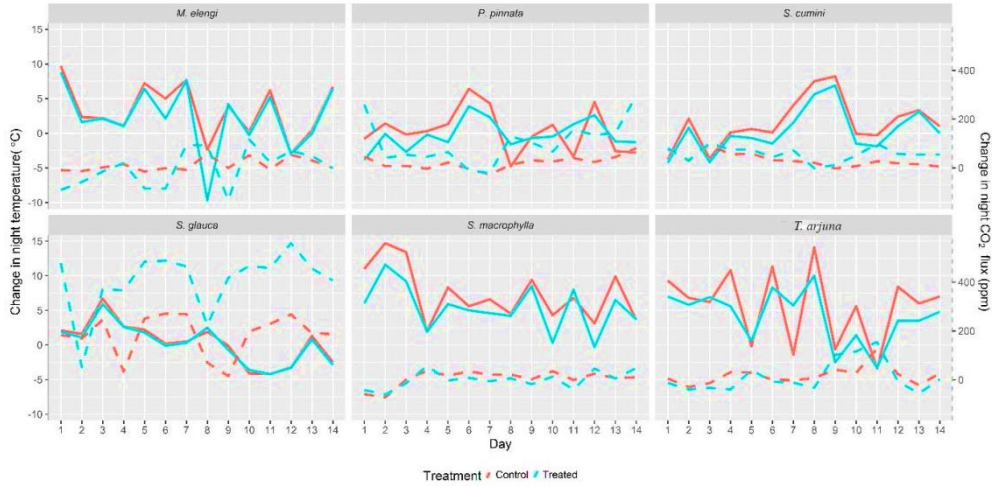


Figure 1.4.3: Changes in night flux of CO_2 with night temperature

Table 1.4.8: Standardization studies on experimental chambers (Temperature in ^{0}C)

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	38.80	45.00	37.30	39.10	-7.70	-17.11	1.80	4.826		40.10	44.20	37.10	40.10	-7.10	-16.06	3.0	8.086
02	39.10	40.40	32.90	36.00	-7.50	-18.56	3.10	9.422		40.10	40.60	33.10	37.00	-7.50	-18.47	3.90	11.78
03	36.00	36.70	34.30	39.60	-2.40	-6.54	5.30	15.45		37.00	37.00	34.30	39.70	-2.70	-7.30	5.40	15.74
04	39.60	41.30	36.50	37.50	-4.80	-11.62	1.00	2.740		39.70	40.30	35.90	37.90	-4.40	-10.92	2.00	5.571
05	37.50	37.70	34.30	43.80	-3.40	-9.02	9.50	27.69		37.90	37.90	34.10	44.40	-3.80	-10.03	10.30	30.20
06	43.80	43.60	36.80	42.80	-6.80	-15.60	6.00	16.30		44.40	44.00	36.30	43.50	-7.70	-17.50	7.20	19.83
07	42.80	44.00	33.70	39.60	-10.3	-23.41	5.90	17.50		43.50	43.90	33.70	39.90	-10.2	-23.23	6.20	18.39
08	39.60	40.00	36.30	44.00	-3.70	-9.25	7.70	21.21		39.90	40.00	35.90	44.00	-4.10	-10.25	8.10	22.56
09	44.00	42.10	37.10	40.20	-5.00	-11.88	3.10	8.356		44.00	43.20	36.80	40.50	-6.40	-14.81	3.70	10.05
10	40.20	39.80	35.90	39.30	-3.90	-9.80	3.40	9.471		40.50	40.10	36.10	40.10	-4.0	-9.98	4.0	11.08
11	39.30	41.40	32.30	39.90	-9.10	-21.98	7.60	23.52		40.10	41.00	32.50	40.10	-8.50	-20.73	7.60	23.38
12	39.90	40.70	33.20	38.50	-7.50	-18.43	5.30	15.96		40.10	40.50	33.30	39.30	-7.20	-17.78	6.0	18.01
13	38.50	39.30	36.40	40.90	-2.90	-7.38	4.50	12.36		39.30	39.50	35.90	40.50	-3.60	-9.11	4.60	12.81
14	40.90	39.60	37.30	39.00	-2.30	-5.81	1.70	4.558		40.50	40.00	36.70	40.20	-3.30	-8.25	3.50	9.537
Avg	40.00	40.82	35.30	40.01	5.521	13.31	4.707	13.52		40.51	40.87	35.12	40.51	5.75	13.89	5.39	15.51
Sd	2.265	2.319	1.781	2.258	2.603	5.818	2.515	7.478		2.122	2.216	1.554	2.121	2.319	5.109	2.305	6.913

Table 1.4.9: Temperature studies (°C) on experimental chambers containing *Terminalia arjuna*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	42.50	41.90	36.20	45.50	-5.70	-13.60	9.30	25.69		38.10	41.90	35.90	42.90	-6.00	-14.32	7.00	19.49
02	45.50	42.70	31.80	38.60	-10.9	-25.53	6.80	21.38		42.90	45.00	31.90	37.70	-13.1	-29.11	5.80	18.18
03	38.60	39.05	32.70	38.90	-6.35	-16.26	6.20	18.96		37.70	37.90	32.50	39.40	-5.40	-14.25	6.90	21.23
04	38.90	39.30	32.90	43.70	-6.40	-16.28	10.80	32.82		39.40	38.70	32.70	38.30	-6.00	-15.50	5.60	17.12
05	43.70	43.00	34.90	34.70	-8.10	-18.84	-0.20	-0.573		38.30	42.30	34.50	35.10	-7.80	-18.44	0.60	1.739
06	34.70	34.30	29.70	41.00	-4.60	-13.41	11.30	38.04		35.10	34.20	29.60	37.90	-4.60	-13.45	8.30	28.04
07	41.00	41.00	40.50	39.10	-0.50	-1.22	-1.40	-3.457		37.90	40.00	31.20	36.90	-8.80	-22.00	5.70	18.26
08	39.10	38.70	28.60	42.70	-10.1	-26.10	14.10	49.30		36.90	38.10	28.50	38.50	-9.60	-25.20	10.0	35.08
09	42.70	42.90	35.30	34.70	-7.60	-17.72	-0.60	-1.700		38.50	41.50	35.30	32.80	-6.20	-14.94	-2.50	-7.082
10	34.70	40.90	34.40	40.00	-6.50	-15.89	5.60	16.27		32.80	40.50	34.60	36.00	-5.90	-14.57	1.40	4.046
11	40.00	38.50	30.30	26.90	-8.20	-21.30	-3.40	-11.22		36.00	39.30	30.40	27.10	-8.90	-22.65	-3.30	-10.85
12	26.90	26.80	29.50	37.90	2.70	10.07	8.40	28.47		27.10	27.00	29.70	33.20	2.70	10.00	3.50	11.78
13	37.90	38.00	32.70	38.70	-5.30	-13.95	6.0	18.34		33.20	37.30	32.50	36.00	-4.80	-12.87	3.50	10.76
14	38.70	39.70	31.50	38.50	-8.20	-20.65	7.0	22.22		36.00	37.50	31.50	36.30	-6.00	-16.00	4.80	15.23
Avg	38.92	39.05	32.93	38.64	6.130	15.05	5.710	18.18		36.42	38.66	32.20	36.29	6.46	15.95	4.09	13.08
Sd	4.641	4.240	3.174	4.526	3.576	9.430	5.256	17.21		3.701	4.275	2.258	3.669	3.502	8.962	3.878	12.66

Table 1.4.10: Temperature (°C) studies on experimental chambers containing *Swietenia macrophylla*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	26.8	27.60	32.00	43.00	4.40	15.94	11.00	34.38		26.6	26.50	32.00	38.00	5.50	20.75	6.00	18.75
02	43.00	44.10	30.20	44.90	-13.9	-31.52	14.70	48.68		38.00	41.40	30.10	41.70	-11.3	-27.29	11.6	38.54
03	44.90	45.30	30.10	43.50	-15.2	-33.55	13.40	44.52		41.70	43.90	30.70	39.90	-13.2	-30.07	9.20	29.97
04	43.50	43.90	31.90	33.90	-12.0	-27.33	2.00	6.270		39.90	42.50	31.60	33.50	-10.9	-25.65	1.90	6.013
05	33.90	35.00	32.40	40.70	-2.60	-7.43	8.30	25.62		33.50	35.00	32.00	37.90	-3.00	-8.57	5.90	18.44
06	40.70	42.00	32.00	37.60	-10.0	-23.81	5.60	17.50		37.90	39.90	32.00	37.00	-7.90	-19.80	5.00	15.63
07	37.60	37.30	33.70	40.30	-3.60	-9.65	6.60	19.58		37.00	37.70	33.40	38.00	-4.30	-11.41	4.60	13.77
08	40.30	41.10	32.60	37.10	-8.50	-20.68	4.50	13.80		38.00	39.20	32.40	36.60	-6.80	-17.35	4.20	12.96
09	37.10	36.70	32.10	41.50	-4.60	-12.53	9.40	29.28		36.60	37.00	32.00	40.50	-5.00	-13.51	8.50	26.56
10	41.50	45.00	32.90	37.20	-12.1	-26.89	4.30	13.07		40.50	43.80	32.70	33.00	-11.1	-25.34	0.30	0.917
11	37.20	38.00	31.50	38.30	-6.50	-17.11	6.80	21.59		33.00	36.60	31.70	39.70	-4.90	-13.39	8.00	25.24
12	38.30	39.40	33.00	36.10	-6.40	-16.24	3.10	9.394		39.70	37.70	33.00	32.70	-4.70	-12.47	-0.30	-0.909
13	36.10	39.10	31.70	41.60	-7.40	-18.93	9.90	31.23		32.70	35.40	31.80	38.30	-3.60	-10.17	6.50	20.44
14	41.60	42.50	33.50	37.10	-9.0	-21.18	3.60	10.75		38.30	40.20	33.30	37.00	-6.90	-17.16	3.70	11.11
Avg	38.75	39.79	32.11	39.49	7.67	17.92	7.37	23.26		36.67	38.34	32.05	37.41	6.29	15.10	5.36	16.96
Sd	4.644	4.788	1.056	3.195	5.131	12.434	3.899	13.030		4.001	4.469	0.914	2.760	4.704	12.40	3.362	10.99

Table 1.4.11: Temperature (°C) studies on experimental chambers containing *Pongamia pinnata*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	44.30	41.70	35.30	34.50	-6.40	-15.35	-0.80	-2.27		41.90	41.10	35.60	31.70	-5.50	-13.38	-3.90	-10.96
02	34.50	35.90	37.30	38.70	1.40	3.90	1.40	3.75		31.70	33.50	36.90	36.80	3.40	10.15	-0.10	-0.27
03	38.70	39.50	36.80	36.60	-2.70	-6.84	-0.20	-0.54		36.80	38.30	36.80	34.10	-1.50	-3.92	-2.70	-7.34
04	36.60	38.30	35.70	36.00	-2.60	-6.79	0.30	0.84		34.10	36.00	35.70	35.50	-0.30	-0.83	-0.20	-0.56
05	36.00	41.50	34.20	35.50	-7.30	-17.59	1.30	3.80		35.50	39.30	35.60	34.30	-3.70	-9.41	-1.30	-3.65
06	35.50	36.70	32.90	39.30	-3.80	-10.35	6.40	19.45		34.30	35.40	33.30	37.20	-2.10	-5.93	3.90	11.71
07	39.30	40.80	36.10	40.40	-4.70	-11.52	4.30	11.91		37.20	39.00	35.80	38.10	-3.20	-8.21	2.30	6.42
08	40.40	41.90	35.80	31.00	-6.10	-14.56	-4.80	-13.41		38.10	40.10	35.70	34.10	-4.40	-10.97	-1.60	-4.48
09	31.00	39.10	36.30	35.80	-2.80	-7.16	-0.50	-1.38		34.10	37.50	36.20	35.50	-1.30	-3.47	-0.70	-1.93
10	35.80	42.40	37.20	38.40	-5.20	-12.26	1.20	3.23		35.50	41.70	36.70	36.20	-5.00	-11.99	-0.50	-1.36
11	38.40	39.50	34.80	31.40	-4.70	-11.90	-3.40	-9.77		36.20	37.90	34.80	36.10	-3.10	-8.18	1.30	3.74
12	31.40	30.70	32.90	37.40	2.20	7.17	4.50	13.68		36.10	37.10	33.10	35.70	-4.00	-10.78	2.60	7.85
13	37.40	38.50	37.70	35.10	-0.80	-2.08	-2.60	-6.90		35.70	37.00	36.90	35.70	-0.10	-0.27	-1.20	-3.25
14	35.10	35.90	36.00	33.20	0.10	0.28	-2.80	-7.78		35.70	35.10	36.80	35.50	1.70	4.84	-1.30	-3.53
Avg	36.74	38.74	35.64	35.95	3.10	7.50	0.310	1.04		35.92	37.79	35.71	35.46	2.08	5.17	0.24	0.54
Sd	3.467	3.168	1.506	2.814	2.934	7.419	3.207	9.292		2.329	2.343	1.238	1.570	2.580	6.784	2.127	6.116

Table 1.4.12: Temperature (°C) studies on experimental chambers containing *Simarouba glauca*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	29.90	30.70	28.00	30.10	-2.70	-8.79	2.10	7.50		29.80	29.90	28.00	29.90	-1.90	-6.35	1.90	6.79
02	30.10	28.70	27.00	28.60	-1.70	-5.92	1.60	5.93		29.90	28.50	27.00	28.10	-1.50	-5.26	1.10	4.07
03	28.60	32.00	28.00	34.70	-4.00	-12.50	6.70	23.93		28.10	31.60	28.00	33.90	-3.60	-11.39	5.90	21.07
04	34.70	36.90	31.00	33.70	-5.90	-15.99	2.70	8.71		33.90	37.30	30.90	33.50	-6.40	-17.16	2.60	8.41
05	33.70	39.10	31.90	34.10	-7.20	-18.41	2.20	6.90		33.50	38.60	31.90	33.70	-6.70	-17.36	1.80	5.64
06	34.10	37.00	32.90	33.10	-4.10	-11.08	0.20	0.61		33.70	36.40	32.90	32.80	-3.50	-9.62	-0.10	-0.30
07	33.10	39.80	33.50	34.00	-6.30	-15.83	0.5	1.49		32.80	39.60	33.70	34.00	-5.90	-14.90	0.30	0.89
08	34.00	37.20	34.00	35.90	-3.20	-8.60	1.9	5.59		34.00	37.20	34.00	36.50	-3.20	-8.60	2.50	7.35
09	35.90	36.00	31.70	31.60	-4.30	-11.94	-0.1	-0.32		36.50	35.90	32.00	31.30	-3.90	-10.86	-0.70	-2.19
10	31.60	42.20	34.90	30.80	-7.30	-17.30	-4.1	-11.75		31.30	41.70	34.60	31.00	-7.10	-17.03	-3.60	-10.40
11	30.80	36.20	33.50	29.30	-2.70	-7.46	-4.2	-12.54		31.00	35.70	33.50	29.30	-2.20	-6.16	-4.20	-12.54
12	29.30	39.70	33.20	30.00	-6.50	-16.37	-3.2	-9.64		29.30	39.40	33.30	30.00	-6.10	-15.48	-3.30	-9.91
13	30.00	34.00	31.00	32.30	-3.00	-8.82	1.3	4.19		30.00	34.10	31.20	32.00	-2.90	-8.50	0.80	2.56
14	32.30	38.50	34.70	32.20	-3.80	-9.87	-2.5	-7.20		32.00	38.10	34.50	31.60	-3.60	-9.45	-2.90	-8.41
Avg	32.01	36.29	31.81	32.17	4.48	12.06	0.36	1.67		31.84	36.00	31.82	31.97	4.180	11.30	0.15	0.93
Sd	2.280	3.782	2.557	2.198	1.830	4.057	3.021	9.766		2.329	3.803	2.528	2.256	1.893	4.324	2.865	9.189

Table 1.4.13: Temperature (°C) studies on experimental chambers containing *Mimusops elengi*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	37.20	48.80	31.60	41.30	-17.2	-35.25	9.70	30.70		36.50	39.80	31.80	40.60	-8.00	-20.10	8.80	27.67
02	41.30	42.00	33.70	36.00	-8.30	-19.76	2.30	6.820		40.60	41.40	33.70	35.30	-7.70	-18.60	1.60	4.75
03	36.00	37.90	33.30	35.50	-4.60	-12.14	2.20	6.610		35.30	35.50	33.40	35.50	-2.10	-5.92	2.10	6.29
04	35.50	37.70	34.90	36.00	-2.80	-7.43	1.10	3.15		35.50	35.40	34.70	35.70	-0.70	-1.98	1.00	2.88
05	36.00	35.50	31.80	39.00	-3.70	-10.42	7.20	22.64		35.70	35.00	32.10	38.50	-2.90	-8.29	6.40	19.94
06	39.00	38.50	32.90	37.90	-5.60	-14.55	5.00	15.20		38.50	39.10	32.90	35.00	-6.20	-15.86	2.10	6.38
07	37.90	38.00	33.40	41.10	-4.60	-12.11	7.70	23.05		35.00	38.30	33.30	40.90	-5.00	-13.05	7.60	22.82
08	41.10	40.70	34.20	31.80	-6.50	-15.97	-2.40	-7.02		40.90	41.00	40.70	31.00	-0.30	-0.73	-9.70	-23.83
09	31.80	32.20	34.30	38.20	2.1.0	6.52	3.90	11.37		31.00	31.70	34.30	38.50	2.60	8.20	4.20	12.24
10	38.20	40.30	32.90	33.10	-7.40	-18.36	0.20	0.61		38.50	38.50	33.00	32.70	-5.50	-14.29	-0.30	-0.91
11	33.10	33.90	30.10	36.30	-3.80	-11.21	6.20	20.60		32.70	32.90	30.50	35.70	-2.40	-7.29	5.20	17.05
12	36.30	36.30	31.50	28.60	-4.80	-13.22	-2.90	-9.21		35.70	36.10	31.80	28.80	-4.30	-11.91	-3.00	-9.43
13	28.60	28.70	29.90	30.30	1.20	4.18	0.40	1.34		28.80	28.70	30.00	29.90	1.30	4.53	-0.10	-0.33
14	30.30	30.60	29.00	35.70	-1.60	-5.23	6.70	23.10		29.90	30.10	29.00	35.40	-1.10	-3.65	6.40	22.07
Avg	35.88	37.22	32.39	35.77	4.83	11.78	3.38	10.64		35.33	35.96	32.94	35.25	3.02	7.78	2.31	7.68
Sd	3.787	5.098	1.798	3.768	4.611	10.149	3.870	12.266		3.705	3.996	2.767	3.690	3.240	8.470	4.827	14.01

Table 1.4.14: Temperature studies (°C) on experimental chambers containing *Syzygium cumini*

Control chamber										CO_2 treated chamber							
Days of experimentation	Temperature morning hours (before air supplementation)	Temperature morning hours (after air supplementation)	Temperature in the evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in the temperature	Night variation (% change)		Temperature morning hours (before CO_2 supplementation)	Temperature morning hours (after CO_2 supplementation)	Temperature evening hours	Temperature in the next day morning	Day variation in temperature	Day variation (% change)	Night variation in temperature	Night variation (% change)
01	36.40	39.50	34.90	31.10	-4.60	-11.65	-3.80	-10.89		36.50	38.10	34.30	30.00	-3.80	-9.97	-4.30	-12.54
02	31.10	32.00	31.00	33.10	-1.00	-3.13	2.10	6.77		30.00	30.50	30.90	31.70	0.40	1.31	0.80	2.59
03	33.10	34.90	33.60	29.90	-1.30	-3.72	-3.70	-11.01		31.70	32.20	33.50	29.30	1.30	4.04	-4.20	-12.54
04	29.90	30.10	29.50	29.60	-0.60	-1.99	0.10	0.34		29.30	29.70	29.80	29.40	0.10	0.34	-0.40	-1.34
05	29.60	30.50	29.50	30.10	-1.00	-3.28	0.60	2.03		29.40	29.80	29.70	29.00	-0.10	-0.34	-0.70	-2.36
06	30.10	30.30	30.60	30.70	0.30	0.99	0.10	0.33		29.00	29.70	30.70	29.20	1.00	3.37	-1.50	-4.89
07	30.70	31.40	32.50	36.50	1.10	3.50	4.00	12.31		29.20	30.10	32.30	33.80	2.20	7.31	1.50	4.64
08	36.50	37.50	31.50	39.00	-6.00	-16.00	7.50	23.81		33.80	35.10	31.50	37.10	-3.60	-10.26	5.60	17.78
09	39.00	39.50	31.80	40.00	-7.70	-19.49	8.20	25.79		37.10	37.70	31.90	38.80	-5.80	-15.38	6.90	21.63
10	40.00	40.50	32.10	32.00	-8.40	-20.74	-0.10	-0.31		38.80	39.60	32.10	30.60	-7.50	-18.94	-1.50	-4.67
11	32.00	33.00	33.30	33.00	0.30	0.91	-0.30	-0.90		30.60	31.10	33.00	31.10	1.90	6.11	-1.90	-5.76
12	33.00	33.70	31.70	34.10	-2.00	-5.93	2.40	7.57		31.10	31.70	31.70	32.70	0	0.00	1.00	3.15
13	34.10	35.00	32.10	35.40	-2.90	-8.29	3.30	10.28		32.70	33.10	32.00	35.10	-1.10	-3.32	3.10	9.69
14	35.40	35.40	32.40	33.40	-3.00	-8.47	1.00	3.09		35.10	35.10	32.20	32.20	-2.90	-8.26	0	0.00
Avg	33.64	34.52	31.89	33.42	2.63	6.95	1.53	4.94		32.45	33.11	31.83	32.14	1.28	3.14	0.31	1.10
Sd	3.392	3.594	1.488	3.298	3.002	7.615	3.480	10.75		3.290	3.430	1.290	3.077	2.979	8.140	3.234	9.999

The humidity experienced within the growth chambers during Standardization studies and studies utilizing respective plant species are depicted in **tables 1.4.15** to **1.4.21**.

Session I

1.4.5 Humidity variations within the chambers associated with Standardization studies

In the CC, there is a slight reduction in the average humidity (morning) before and after the air supplementation. A reduction in humidity is also observed in the TC after CO_2 supplementation. Evening humidity is higher in the TC compared to the CC. The percentage change in the day variation in the CC and the TC is 18.07±14.27 and 32.41±18.08, respectively. The percentage change in the night variation in the CC and TC is respectively -10.29± 9.18 and -20.22±7.92 **(Table 1.4.15)**.

Session II

1.4.6 Humidity variations within the chambers having plant growth

A slight decrease in the average humidity is noticed in the CC and TC with *T. arjuna* after air and CO_2 supplementation. A meager increase in humidity is observed in the TC in the evening. The percentage variation in the day in the CC and the TC is observed to be 9.28±8.28 and 4.8±9.40 while in the night is -3.77±6.34 and -2.16±3.90, respectively **(Table 1.4.16)**.

Reduction in humidity is also observed in the CC and TC with *S. macrophylla* after air and CO_2 supplementation. In both the CC and TC, evening humidity is observed to be 99%. The percentage change in the day in humidity with *S. macrophylla* is 5.88±7.65 in the CC and 3.23±5.56 in the TC. The percentage change at night is -2.74±5.22 in the CC and -1.23±2.75 in the TC **(Table 1.4.17)**.

In the CC with *P. pinnata,* a slight reduction in humidity is noticed after air supplementation while in the TC, humidity has increased slightly. Humidity in the evening is higher in the TC compared to the CC. The percentage change in the day variation in humidity is 4.27±15.22 in the CC and 4.84±17.57 in the TC. Similarly, the percentage change in the night variation in humidity is -2.13± 28.72 in the CC and 0.46±1.72 in the TC, which is depicted in **Table 1.4.18**.

In the CC and TC with *S. glauca,* humidity decreased with air and CO_2 supplementation. Humidity increased in the TC compared to the CC in the evening. The percentage day change

in humidity is 5.04±7.81 in the CC and 4.36±5.98 in the TC. The percentage change in the night humidity is 2.69±3.42 in the CC. There is no change in night variation in the TC **(Table 1.4.19)**.

In the CC and TC with *M. elengi,* humidity decreased with air and CO_2 supplementation. Humidity increased in the TC compared to CC in the evening. The percentage variation in the day humidity is 9.42±18.74 in the CC and 6.05±8.74 in the TC. The percentage change in the night humidity is -1.59±6.53 in the CC and -1.06±7.38 in the TC, and is shown in **table 1.4.20**.

In the CC and TC with *S. cumini,* humidity decreased with air and CO_2 supplementation. There is only a considerable change in humidity in the TC in the evening compared to the CC. The percentage change in the day humidity is 3.32±8.45 in the CC and 2.77±7.15 in the TC. Similarly, the percentage change in the night is 0.99±3.69 in the CC and 0.46±1.72 in the TC **(Table 1.4.21)**.

Table 1.4.15: Standardization studies on experimental chambers (Humidity in %)

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)	Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours (after CO_2 supplementation)	Humdity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	56.00	40.00	51.00	53.00	11.00	27.50	2.00	3.92	53.00	54.00	59.00	54.00	5.00	9.26	-5.00	-8.47
02	53.00	50.00	62.00	59.00	12.00	24.00	-3.00	-4.84	54.00	52.00	73.00	59.00	21.00	40.38	-14.00	-19.18
03	59.00	59.00	60.00	54.00	1.00	1.69	-6.00	-10.00	59.00	59.00	60.00	56.00	1.00	1.69	-4.00	-6.67
04	54.00	50.00	54.00	53.00	4.00	8.00	-1.00	-1.85	56.00	55.00	67.00	58.00	12.00	21.82	-9.00	-13.43
05	53.00	52.00	59.00	45.00	7.00	13.46	-14.00	-23.73	58.00	58.00	71.00	47.00	13.00	22.41	-24.00	-33.80
06	45.00	43.00	51.00	46.00	8.00	18.60	-5.00	-9.80	47.00	46.00	64.00	49.00	18.00	39.13	-15.00	-23.44
07	46.00	43.00	62.00	51.00	19.00	44.19	-11.00	-17.74	49.00	48.00	74.00	55.00	26.00	54.17	-19.00	-25.68
08	51.00	50.00	54.00	44.00	4.00	8.00	-10.00	-18.52	55.00	40.00	66.00	47.00	26.00	65.00	-19.00	-28.79
09	44.00	45.00	53.00	52.00	8.00	17.78	-1.00	-1.89	47.00	43.20	64.00	55.00	20.80	48.15	-9.00	-14.06
10	52.00	52.00	59.00	54.00	7.00	13.46	-5.00	-8.47	55.00	55.00	68.00	56.00	13.00	23.64	-12.00	-17.65
11	54.00	49.00	70.00	52.00	21.00	42.86	-18.00	-25.71	56.00	54.00	79.00	56.00	25.00	46.30	-23.00	-29.11
12	52.00	51.00	66.00	54.00	15.00	29.41	-12.00	-18.18	56.00	55.00	78.00	59.00	23.00	41.82	-19.00	-24.36
13	54.00	52.00	50.00	50.00	-2.00	-3.85	0.00	0.00	59.00	58.00	69.00	57.00	11.00	18.97	-12.00	-17.39
14	50.00	51.00	55.00	51.00	4.00	7.84	-4.00	-7.27	57.00	57.00	69.00	54.50	12.00	21.05	-14.50	-21.01
Avg	51.64	49.07	57.57	51.29	8.50	18.07	-6.29	-10.29	54.36	52.44	68.64	54.46	16.20	32.41	-14.18	-20.22
Sd	4.22	4.86	6.02	4.03	6.56	14.27	5.88	9.18	4.03	5.89	6.01	4.01	7.86	18.08	6.18	7.92

Table 1.4.16: Humidity studies (%) on experimental chambers containing *Terminalia arjuna*

Control chamber										CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)		Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours (after CO_2 supplementation)	Humdity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	77.00	80.00	99.00	85.00	19.00	23.75	-14.00	-14.14		75.00	75.00	99.00	89.00	24.00	32.00	-10.00	-10.10
02	85.00	81.00	99.00	99.00	18.00	22.22	0.00	0.00		89.00	85.00	99.00	99.00	14.00	16.47	0.00	0.00
03	99.00	90.50	99.00	91.00	8.50	9.39	-8.00	-8.08		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
04	91.00	94.00	99.00	86.00	5.00	5.32	-13.00	-13.13		99.00	91.00	99.00	89.00	8.00	8.79	-10.00	-10.10
05	86.00	82.00	99.00	99.00	17.00	20.73	0.00	0.00		89.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00
06	99.00	99.00	99.00	92.00	0.00	0.00	-7.00	-7.07		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
07	92.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
08	99.00	93.00	99.00	88.00	6.00	6.45	-11.00	-11.11		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
09	88.00	84.00	91.00	99.00	7.00	8.33	8.00	8.79		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	88.00	99.00	99.00	11.00	12.50	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	95.00	0.00	0.00	-4.00	-4.04		99.00	99.00	99.00	92.00	0.00	0.00	-7.00	-7.07
14	95.00	89.00	99.00	95.00	10.00	11.24	-4.00	-4.04		92.00	99.00	99.00	96.00	0.00	0.00	-3.00	-3.03
Avg	93.36	90.54	98.43	94.64	7.89	9.28	-3.79	-3.77		95.36	95.07	99.00	96.86	3.93	4.80	-2.14	-2.16
Sd	7.09	6.97	2.14	5.30	6.71	8.28	6.17	6.34		7.01	7.34	0.00	3.86	7.34	9.40	3.86	3.90

Table 1.4.17. Humidity studies (%) on experimental chambers containing *Swietenia macrophylla*

Control chamber										CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)		Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours (after CO_2 supplementation)	Humdity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	99.00	99.00	99.00	85.00	0.00	0.00	-14.00	-14.14		99.00	99.00	99.00	92.00	0.00	0.00	-7.00	-7.07
02	85.00	82.00	99.00	85.00	17.00	20.73	-14.00	-14.14		92.00	87.00	99.00	91.00	12.00	13.79	-8.00	-8.08
03	85.00	84.00	99.00	92.00	15.00	17.86	-7.00	-7.07		91.00	87.00	99.00	99.00	12.00	13.79	0.00	0.00
04	92.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00		99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00
05	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
06	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
07	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
08	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
09	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	84.00	99.00	99.00	15.00	17.86	0.00	0.00		99.00	89.00	99.00	99.00	10.00	11.24	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	95.00	99.00	96.00	4.00	4.21	-3.00	-3.03		99.00	99.00	99.00	97.00	0.00	0.00	-2.00	-2.02
Avg	96.50	93.93	99.00	96.29	5.07	5.88	-2.71	-2.74		97.93	96.14	99.00	97.79	2.86	3.23	-1.21	-1.23
Sd	5.21	6.39	0.00	5.17	6.39	7.65	5.17	5.22		2.73	4.88	0.00	2.72	4.88	5.56	2.72	2.75

Table 1.4.18: Humidity studies (%) on experimental chambers containing *Pongamia pinnata*

Control chamber										CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)		Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours (after CO_2 supplementation)	Humdity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	46.00	60.00	89.00	99.00	29.00	48.33	10.00	11.24		50.00	60.00	99.00	99.00	39.00	65.00	0.00	0.00
02	99.00	99.00	84.00	91.00	-15.00	-15.15	7.00	8.33		99.00	99.00	93.00	99.00	-6.00	-6.06	6.00	6.45
03	91.00	87.00	87.00	99.00	0.00	0.00	12.00	13.79		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
04	99.00	93.00	93.00	99.00	0.00	0.00	6.00	6.45		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
05	99.00	85.00	93.00	99.00	8.00	9.41	6.00	6.45		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
06	99.00	99.00	99.00	94.00	0.00	0.00	-5.00	-5.05		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
07	94.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
08	99.00	90.00	99.00	99.00	9.00	10.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
09	99.00	90.00	93.00	99.00	3.00	3.33	6.00	6.45		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	78.00	89.00	99.00	11.00	14.10	10.00	11.24		99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	89.00	99.00	-10.00	-10.10	10.00	11.24		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	89.00	98.00	-10.00	-10.10	-89.00	-100.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Avg	94.29	90.50	92.93	98.00	2.43	4.27	-1.93	-2.13		95.50	95.64	98.57	99.00	2.93	4.84	0.43	0.46
Sd	14.11	10.99	5.27	2.52	10.88	15.22	25.56	28.72		13.10	10.48	1.60	0.00	10.75	17.57	1.60	1.72

Table 1.4.19: Humidity studies (%) on experimental chambers containing *Simarouba glauca*

Control chamber										CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)		Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours (after CO_2 supplementation)	Humdity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
02	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
03	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
04	99.00	84.00	99.00	99.00	15.00	17.86	0.00	0.00		99.00	86.00	99.00	99.00	13.00	15.12	0.00	0.00
05	99.00	85.00	99.00	99.00	14.00	16.47	0.00	0.00		99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00
06	99.00	93.00	93.00	99.00	0.00	0.00	6.00	6.45		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
07	99.00	88.00	95.00	99.00	7.00	7.95	4.00	4.21		99.00	92.00	99.00	99.00	7.00	7.61	0.00	0.00
08	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
09	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	79.00	91.00	99.00	12.00	15.19	8.00	8.79		99.00	85.00	99.00	99.00	14.00	16.47	0.00	0.00
11	99.00	99.00	92.00	99.00	-7.00	-7.07	7.00	7.61		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	92.00	95.00	99.00	3.00	3.26	4.00	4.21		99.00	95.00	99.00	99.00	4.00	4.21	0.00	0.00
13	99.00	88.00	99.00	99.00	11.00	12.50	0.00	0.00		99.00	91.00	99.00	99.00	8.00	8.79	0.00	0.00
14	99.00	95.00	93.00	99.00	-2.00	-2.11	6.00	6.45		99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Avg	99.00	92.29	96.50	99.00	4.21	5.04	2.50	2.69		99.00	95.14	99.00	99.00	3.86	4.36	0.00	0.00
Sd	0.00	6.62	3.16	0.00	6.70	7.81	3.16	3.42		0.00	5.19	0.00	0.00	5.19	5.98	0.00	0.00

Table 1.4.20: Humidity studies (%) on experimental chambers containing *Mimusops elengi*

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)	Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours in (after CO_2 supplementation)	Humdity evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	69.00	54.00	91.00	78.00	37.00	68.52	-13.00	-14.29	84.00	78.00	99.00	83.00	21.00	26.92	-16.00	-16.16
02	78.00	75.00	91.00	92.00	16.00	21.33	1.00	1.10	83.00	81.00	95.00	99.00	14.00	17.28	4.00	4.21
03	92.00	94.00	95.00	99.00	1.00	1.06	4.00	4.21	99.00	93.00	99.00	99.00	6.00	6.45	0.00	0.00
04	99.00	99.00	91.00	99.00	-8.00	-8.08	8.00	8.79	99.00	99.00	95.00	99.00	-4.00	-4.04	4.00	4.21
05	99.00	93.00	99.00	90.00	6.00	6.45	-9.00	-9.09	99.00	94.00	99.00	91.00	5.00	5.32	-8.00	-8.08
06	90.00	88.00	99.00	99.00	11.00	12.50	0.00	0.00	91.00	89.00	99.00	99.00	10.00	11.24	0.00	0.00
07	99.00	96.00	99.00	86.00	3.00	3.13	-13.00	-13.13	99.00	93.00	99.00	88.00	6.00	6.45	-11.00	-11.11
08	86.00	86.00	95.00	99.00	9.00	10.47	4.00	4.21	88.00	86.00	86.00	99.00	0.00	0.00	13.00	15.12
09	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	85.00	99.00	99.00	14.00	16.47	0.00	0.00	99.00	86.00	99.00	99.00	13.00	15.12	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	95.00	0.00	0.00	-4.00	-4.04	99.00	99.00	99.00	96.00	0.00	0.00	-3.00	-3.03
Avg	93.29	90.36	96.71	95.14	6.36	9.42	-1.57	-1.59	95.43	92.43	97.50	96.29	5.07	6.05	-1.21	-1.06
Sd	9.54	12.74	3.41	6.49	10.98	18.74	6.19	6.53	6.12	7.31	3.61	5.17	7.12	8.74	6.96	7.38

Table 1.4.21: Humidity studies (%) on experimental chambers containing *Syzygium cumini*

Control chamber									CO_2 treated chamber							
Days of experimentation	Humidity in morning hours (before air supplementation)	Humidity in morning hours (after air supplementation)	Humidity in the evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in the humidity	Night variation (% change)	Humidity in morning hours (before CO_2 supplementation)	Humidity in morning hours (after CO_2 supplementation)	Humdity in evening hours	Humidity in the next day morning	Day variation in humidity	Day variation (% change)	Night variation in humidity	Night variation (% change)
01	77.00	70.00	87.00	99.00	17.00	24.29	12.00	13.79	74.00	76.00	93.00	99.00	17.00	22.37	6.00	6.45
02	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
03	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
04	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
05	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
06	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
07	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
08	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
09	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
10	99.00	81.00	99.00	99.00	18.00	22.22	0.00	0.00	99.00	85.00	99.00	99.00	14.00	16.47	0.00	0.00
11	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
12	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
13	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
14	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00	99.00	99.00	99.00	99.00	0.00	0.00	0.00	0.00
Avg	97.43	95.64	98.14	99.00	2.50	3.32	0.86	0.99	97.21	96.36	98.57	99.00	2.21	2.77	0.43	0.46
Sd	5.88	8.80	3.21	0.00	6.36	8.45	3.21	3.69	6.68	6.95	1.60	0.00	5.66	7.15	1.60	1.72

CO_2 flux and temperature are shown to be negatively correlated in all the plants except *S. macrophylla*. CO_2 flux and humidity are found to be positively correlated in all the plants except *S. macrophylla*. In the present study in *T. arjuna*, a strong negative correlation was found between the day flux of CO_2 and temperature after CO_2 supplementation, and a strong positive correlation was found between CO_2 flux and humidity. The correlation between temperature and humidity is found to be negative in all the plants under elevated CO_2 conditions and is significant. A significant correlation is represented by * in the figure. (**Figure 1.4.4 to Figure 1.4.9**).

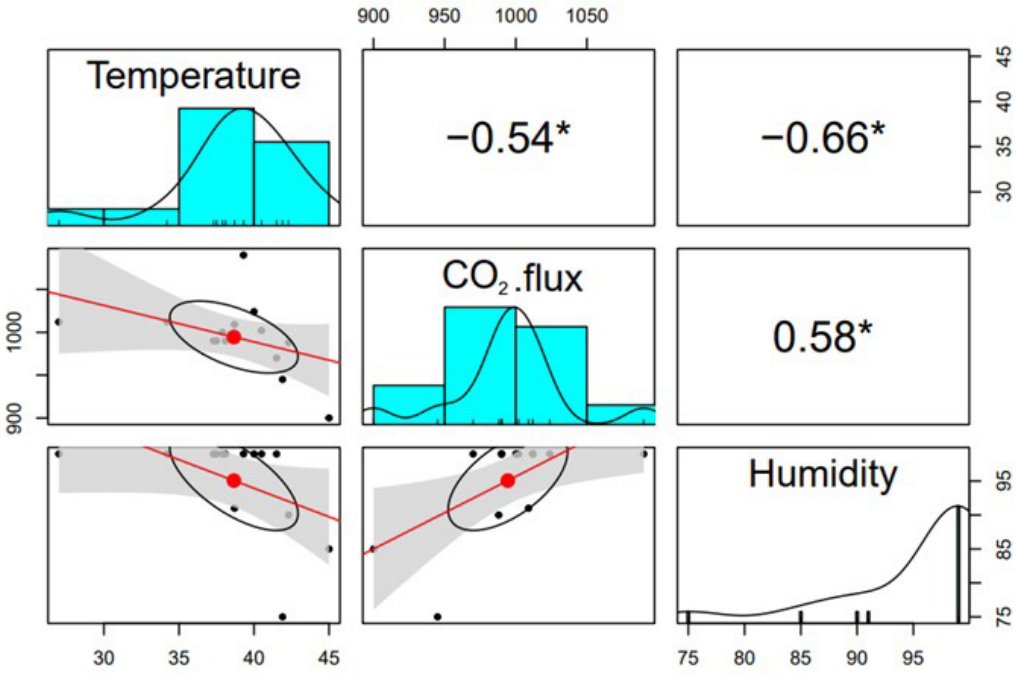


Figure 1.4.4: Correlation of microclimatic variables after elevated CO_2 condition in *Terminalia arjuna*

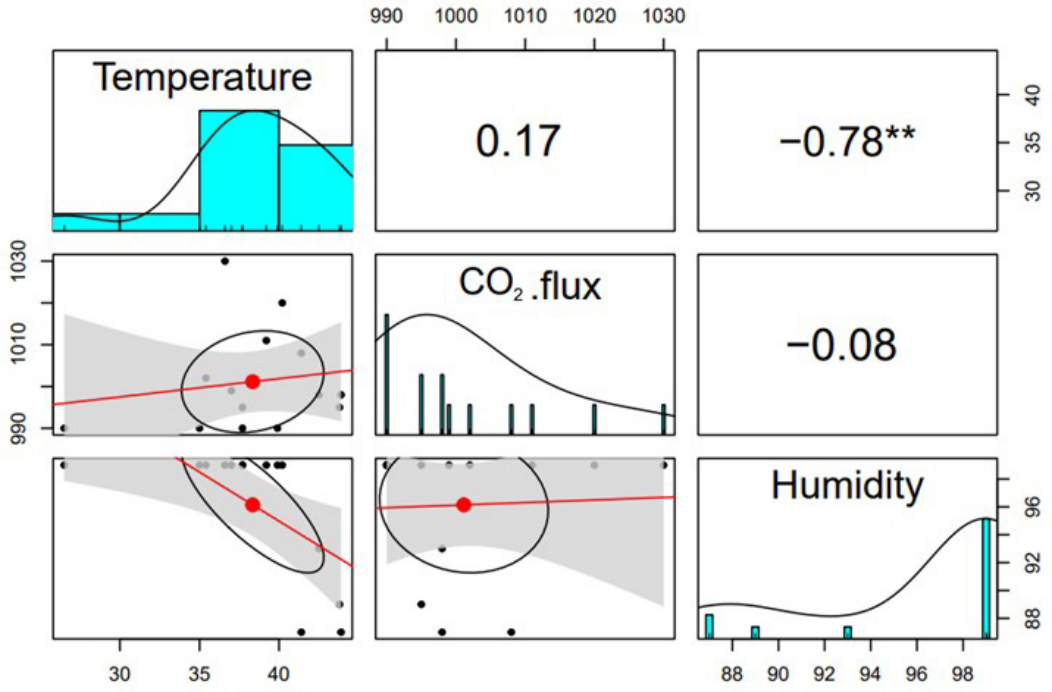


Figure 1.4.5: Correlation of microclimatic variables after elevated CO_2 condition in *Swietenia macrophylla*

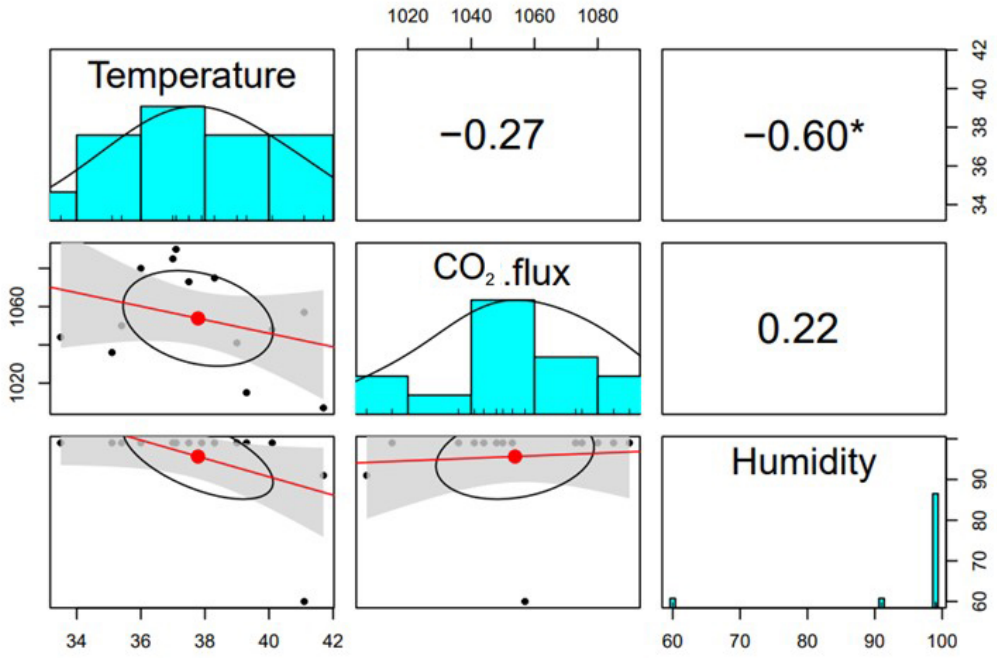


Figure 1.4.6: Correlation of microclimatic variables after elevated CO_2 condition in *Pongamia pinnata*

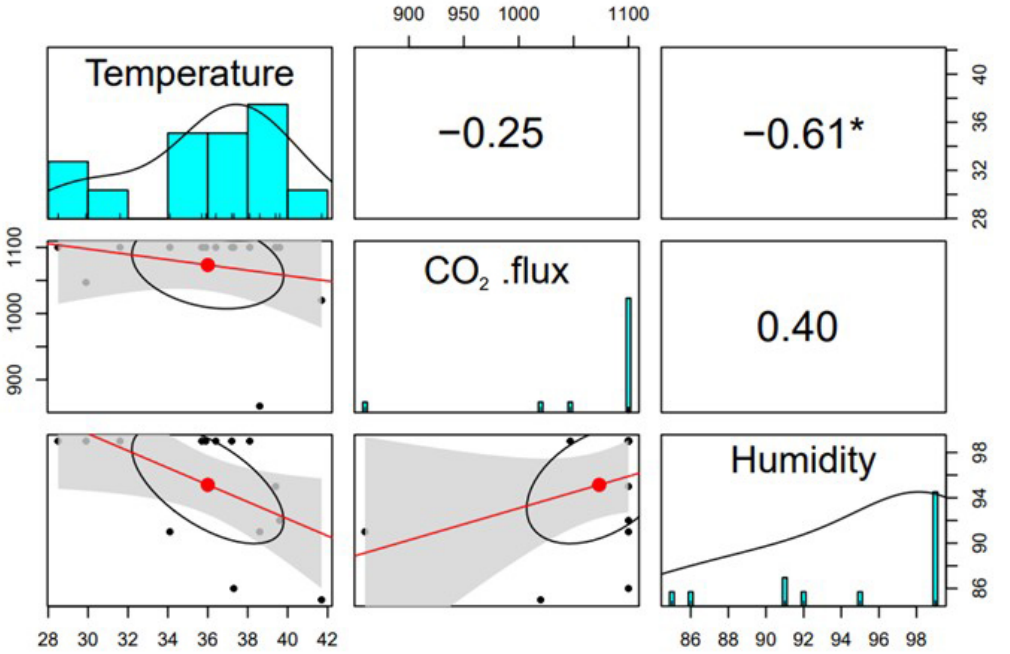


Figure 1.4.7: Correlation of microclimatic variables after elevated CO_2 condition in *Simarouba glauca*

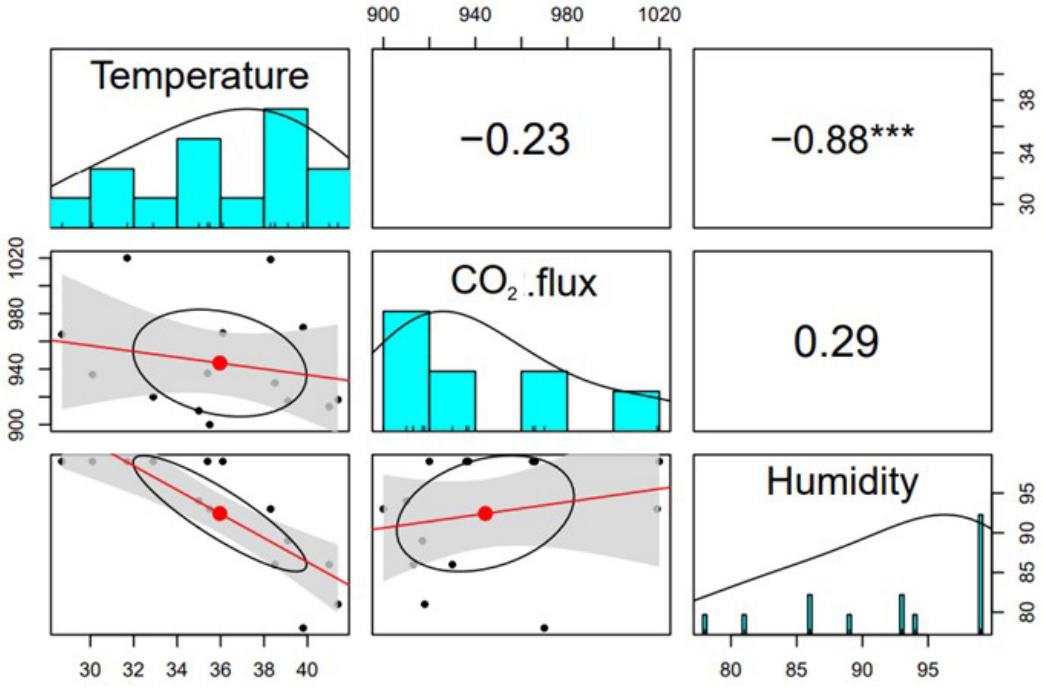


Figure 1.4.8: Correlation of microclimatic variables after elevated CO_2 condition in *Mimusops elengi*

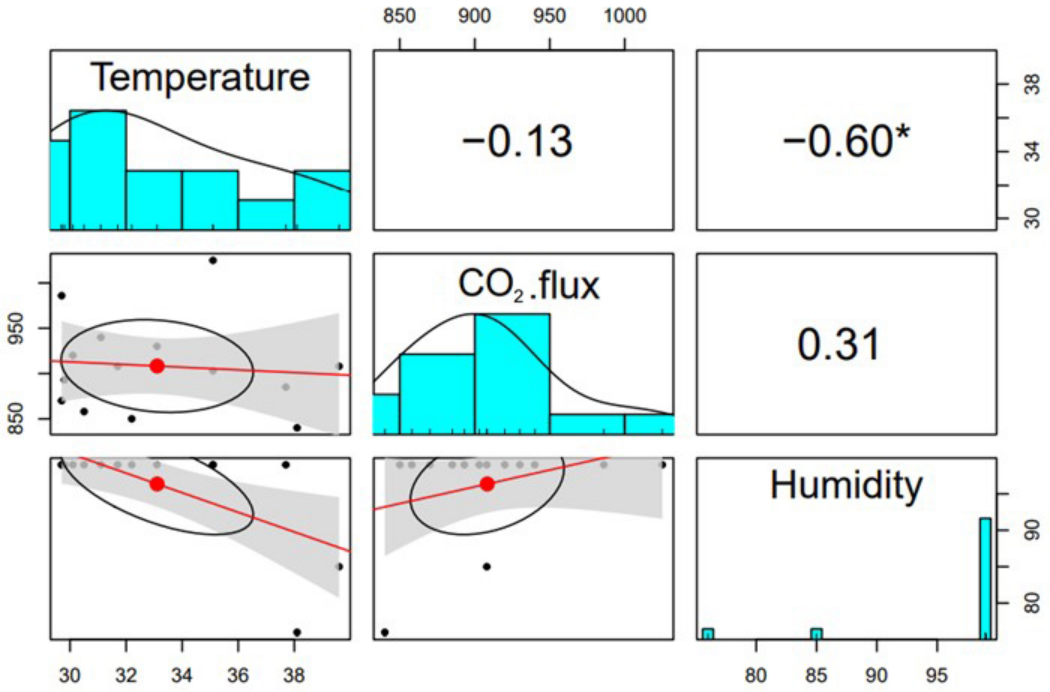


Figure 1.4.9: Correlation of microclimatic variables after elevated CO_2 condition in *Syzygium cumini*

1.5 DISCUSSION

Standardization studies

The prime objective of this study was to assess the CO_2 assimilation efficiencies of selected tree species using controlled growth chambers. Attempts were also carried out to assess the changes in microclimatic conditions within the chamber brought about by the growth of plants under varying levels of Carbon dioxide supply.

Through extensive literature collection and review, an inventory of the avenue trees ideal for tropical climatic conditions has been prepared and details about their multiplication and maintenance have been worked out. Accordingly, six tree species have been selected and a sufficient number of saplings have been raised and retained in the nursery for acclimatization. One-and-a-half-year-old saplings of uniform size have been used for the present study. Meantime, two growth chambers of uniform size (volume 6.32 m^3) have been installed in the polyhouse, each with the facility for supplying ambient air, and air- CO_2 mixture at varying dosages. Facilities have also

been arranged for monitoring the CO_2 concentration (ppm), humidity (%), and temperature (^{0}C) associated with the chamber.

An experiment was carried out in the first phase to standardize the growth chamber, which was to assess the retention pattern of CO_2 within the chamber under ideal conditions. This was accomplished through the supply of ambient air in the control chamber (CC) and an enhanced level of CO_2 in the treatment chamber (TC) at specific times of the day and assessing their natural dissipation/assimilation rate on a day and night time scale, for 15 days. The temperature and humidity associated with both chambers were also assessed along with the monitoring of CO_2.

The results of the standardization studies (Gross assimilation) have been used in the assessment of the CO_2 assimilation studies undertaken in the same growth chamber using saplings of selected tree species (Net assimilation). The changes in the flux (day and night) of CO_2 and other microclimatic conditions (temperature and humidity) attributed by the plants are worked out in Chapter I and those concerning their growth and biochemical attributes in Chapter II.

At the start of the experiment, it is assumed that the temperature, humidity, and pressure in both the CC and TC are identical. The range of CO_2 received by the CC during 15 days of experimentation ranged from 602 to 694 ppm with a mean value of 665 ppm (± 23.26) and TC from 996 to 1045 ppm with a mean value of 1018.4 (± 14.22). The day assimilation in CC ranged from -63 to 15 ppm with a mean value of -30.71 ± 17.94 and night assimilation from 20 to 58 ppm with a mean value of 37.43 ± 11.26. Similarly, the day assimilation in TC ranged from -56 to -109 ppm with a mean value of -77.07 ± 16.83, and night assimilation from 2 to 61 ppm with a mean value of 24.71 ± 15.08. In the standardization study, a greater dissipation and resultant reduction of CO_2 was observed in the TC (-7.55 ± 1.56%) compared to the CC (-4.54 ± 2.66%) during the daytime and higher dissipation in CC (5.91 ± 1.80%) than the TC (2.62 ± 1.61%) during the night.

Several reasons can be attributed to the higher reduction in CO_2 in TC than in CC during daytime and are linked mostly to the physical as well as chemical properties of the gaseous molecule. Accordingly, the higher difference in CO_2 assimilation in

TC compared to the CC may be due to the diffusion of CO_2 gas from the treatment chamber to the outer atmosphere owing to higher pressure differences (Ahmadpour *et al*., 2014). The difference in concentration aids CO_2 molecules to move from an area of higher concentration to a lower concentration and the solubility coefficient of Carbon dioxide increases at high temperatures. The higher temperature (**40.87 deg cel.**) inside the TC might have been attributed to the kinetic energy of gas molecules leading to their higher diffusion. The difference in concentration might have acted as a driving force for CO_2 molecules to move from a region of higher concentration to a lower one (Poudel and Dunn, 2017). Being a greenhouse gas, Carbon dioxide absorbs more of the reflected radiation and enhances the greenhouse effect by increasing the temperature inside the chamber (Zhong and Haigh, 2013). Moreover, the higher temperature retained in the chamber can also be attributed to the heat released by the Carbon dioxide gas as a result of the increased collision of molecules under high pressure (Charriere *et al*., 2010). However, in CC, CO_2 diffuses slowly and proper air circulation helps to distribute CO_2 evenly throughout. This can be the reason for a comparatively lower reduction in CO_2 in the CC during the daytime.

The polyvinyl chloride sheets which are normally used for the construction of similar chambers are permeable to CO_2 gas (Mohagheghian *et al*., 2014) and the reduction of CO_2 in the present study might also be due to the permeability of CO_2 gas from TC to the outer atmosphere (Ahmadpour *et al*., 2014). Carbon dioxide permeation properties of polyvinyl chloride also substantiate the findings of the present study as PVC pipes used for similar experiments are reported to be permeable to CO_2 (Sadeghi *et al*., 2008). Thus, from various literature, it is proved that the polyvinyl chloride pipes and sheets used for the construction of chambers are permeable to CO_2 gas.

Though the day flux in CO_2 in CC and TC showed a marginal decrease with 4.54% and 7.55% respectively, the night flux showed a reversal with an increase in CO_2 in CC (5.91 ± 1.80%) and TC (2.62 ± 1.61%). At increased temperature, CO_2 gas disperses more quickly due to lesser density and increased diffusion which might be the reason for the reduction of day flux in TC (Messerli *et al*., 2015). A reversal of the above can happen at night, which is evident in CC and TC with a marginal increase in CO_2 during night. There can also be the attribution of the building

materials (Polyvinyl chloride pipes, and sheets) to this process. The night flux in the CC was found to be slightly higher than that in the TC, which can also be attributed to the differences in pressure-mediated diffusion of the gas.

In the present study from **Figure 1.5.1** FTIR peak at 2929 cm^{-1} is assigned to CH stretching in polyvinyl chloride. Also, the FTIR peak at 1256 cm^{-1} corresponds to CH rocking vibration and 836 cm^{-1} is assigned to CCl stretching. The result in the present study corroborates the findings of Ramesh *et al*. (2007) where for polyvinyl chloride sheets CH stretching mode is observed between 2890-2958 cm^{-1}, CH rocking mode at 1240- 1257 cm^{-1} and CCl stretching mode at 834 cm^{-1}. A small change in the FTIR spectrum compared to pure polyvinyl chloride might be due to addition of plasticizers in the sample to get flexibility (Ahmadpour *et al*., 2014).

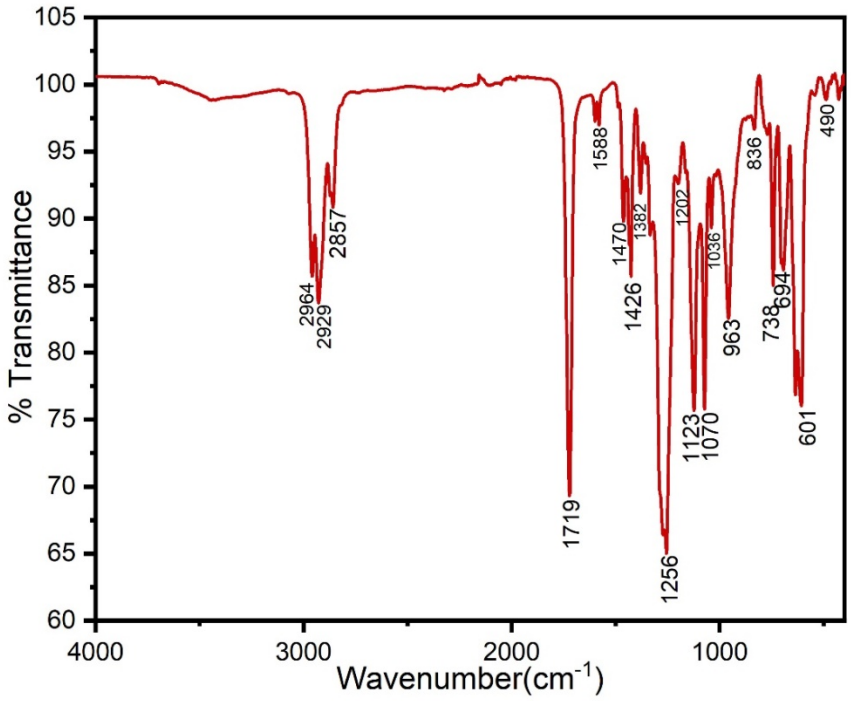


Figure 1.5.1. FTIR spectrum of polyvinyl chloride sheet

Carbon dioxide assimilation studies using tree species

The results of the standardization study have been taken into account for estimating the actual day and night flux (Net flux) of CO_2 for each species maintained in CC and TC for experimentation. The Net flux in CO_2 (Day and Night) for each species

is calculated by subtracting the standardization values from the respective gross flux values. The gross and net values (in ppm) obtained in this regard are depicted in the following table. The amount of CO_2 uptake by each plant is estimated as its assimilation efficiency. A negative sign depicts the reduction in CO_2 within the chamber and the positive sign is representative of the CO_2 attribution.

CO_2 fluxes (ppm)	CC (DF)	TC (DF)	CC (NF)	TC (NF)
Standardization (Control)	-30.71	--	37.43	--
Standardization (Treated)	--	-77.07	--	24.71
T. arjuna (G)	-2.857	-473.4	17.93	12.36
T. arjuna (N)	27.853	-396.33	-19.5	-12.35
S. macrophylla (G)	1.07	-517.5	5.5	-0.07
S. macrophylla (N)	31.78	-440	-31.93	-24.78
P. pinnata (G)	-4.5	-467.5	22.07	104.8
P. pinnata (N)	26.21	-390.43	-15.36	80.09
S. glauca (G)	-179.6	-425.2	184.4	406.6
S. glauca (N)	-148.89	-348	146.97	381.89
M. elengi (G)	-0.85	-258.8	11.21	-0.643
M. elengi (N)	29.86	-181.73	-26.22	-25.353
S. cumini (G)	-32.57	-265.9	33.43	57.71
S. cumini (N)	-1.86	-188.83	-4	33

Where: CC is the Control Chamber; TC is the Treated Chamber; DF is Day Flux; NF is Night Flux; (G) is Gross assimilation and (N) is Net assimilation.

The gross day and night flux inside the TC for *T. arjuna* is -473.4 ppm and 12.36 ppm, respectively. The day flux for the standardization study inside the TC is -77.07 ppm and the night flux is 24.7 ppm. Accordingly, the net flux (day) for *T. arjuna* is -396.33 ppm and the night flux is -12.35. Similarly, for *S. macrophylla* the day flux

of -440.43ppm and the night flux of -24.78ppm is noticed. *P. pinnata* showed a net day flux of -390.43 ppm and a night flux (net) of 80.09 ppm. The net day flux of *S. glauca* is -348 ppm and that of the night flux (net) is 381.89 ppm. *M. elengi* showed a net day flux of -181.73ppm and a night flux (net) of -25.35ppm. The net day flux of *S. cumini* is -188.83ppm and that of the night flux (net) is 33ppm.

Likewise, the relative efficiency of the plants in assimilating Carbon dioxide has also been worked out in terms of percentage and is depicted in the following table. The estimation has been carried out by calculating the gross day flux (%) and night flux (%) and subtracting the standardization values (%) from it.

Percentage change	CC (DF)	TC (DF)	CC (NF)	TC (NF)
Standardization (Control)	-4.54	--	5.91	--
Standardization (Treated)		7.55		2.62
T. arjuna (G)	-0.38	-47.6	3.416	2.296
T. arjuna (N)	4.16	-55.15	-2.494	-0.324
S. macrophylla (G)	0.713	-51.68	1.495	0.063
S. macrophylla (N)	5.253	-59.23	-4.415	-2.557
P. pinnata (G)	-.775	-44.32	4.454	18.15
P. pinnata (N)	3.765	-51.87	-1.456	15.53
S. glauca (G)	-23.78	-39.48	35.3	66.48
S. glauca (N)	-19.24	-47.03	29.39	63.86
M. elengi (G)	-0.019	-27.29	2.236	0.501
M. elengi (N)	4.521	-34.84	-3.674	-2.119
S. cumini (G)	-5.064	-28.99	6.007	8.93
S. cumini (N)	-0.524	-36.54	0.097	6.31

Where: CC is the Control Chamber; TC is the Treated Chamber; DF is Day Flux; NF is Night Flux; (G) is Gross assimilation and (N) is Net assimilation.

Accordingly, the gross day and night flux in the TC for *T. arjuna* is -47.6% and 2.296%, respectively. Likewise, the percentage of day and night flux (net) in *T. arjuna* is -55.15% and -0.324%, respectively. *S. macrophylla* showed a net day flux of -59.23% and a night flux of -2.557%. The net day flux in *P. pinnata* is -51.87% and that of night flux is 15.53%. *S. glauca* showed a net day flux of -47.03% and a night flux of 63.86%. *M. elengi* showed a net day flux of -34.84% and a net night flux of -2.119%. The net day flux (%) of *S. cumini* is -36.54 and that of the night flux (net) is 6.31.

Considering various attributes, it has been noticed that the assimilation of Carbon dioxide was higher in *S. macrophylla*, followed by *T. arjuna*, *P. pinnata*, *S. glauca*, *S. cumini*, and *M. elengi*. From the gross value of night flux in TC, the attribution of Carbon dioxide to the system was higher with *S. glauca* (66.48 ppm) followed by *P. pinnata* (18.15 ppm), *S. cumini* (8.93), *T. arjuna* (2.29 ppm), *M. elengi* (0.501), and *S. macrophylla* (0.063). Considering the performances of the plants under study concerning the day and night fluxes, it has been noticed that *S. macrophylla*, followed by *T. arjuna*, are effective in carbon assimilation, with higher CO_2 assimilation during daytime and lower releases during night time. Species like *P. pinnata* and *S. glauca*, though significant in terms of daytime CO_2 assimilation, their resultant release of CO_2 during nighttime was higher, showing lesser performances in the perspective of Carbon sequestration.

The changes in the day flux and night flux of CO_2 in the TC compared to CC are assumed to be due to the adaptive responses of the respective species under experimentation. Various factors contribute to the CO_2 assimilation efficiency and are mostly linked with the genetic setup, physiological processes, and age of the plants. Most C3 plants respond to elevated levels of CO_2 by increased net photosynthesis (Hawkins *et al*., 2008). According to Prasath *et al*. (2016), a higher photosynthetic rate depends on the species with the best carbon sequestration capacity. The study of Sekhar *et al*. (2023) revealed that the photosynthetic efficiency and thereby carbon sequestration efficiency of *Conocarpus erectus* increased under elevated CO_2 conditions. According to Da Silva Fortirer *et al*. (2023), the higher rate of photosynthesis in response to elevated CO_2 and high biomass accumulation is attributed to the long life span of the trees and their ability

to allocate resources for growth and storage. In an elevated CO_2 experiment conducted in grapevines, the net CO_2 assimilation rate and photosynthetic rate are higher in elevated CO_2 compared to the control. One of the findings of this study is that when plants are exposed to elevated CO_2 for weeks or months, photosynthetic acclimation takes place which might be due to source-sink relationships (Goncalves *et al*., 2009).

The study of the photosynthetic mechanism of the plants does not come under the scope of this research. However, the present study revealed that CO_2 assimilation is higher in *S. macrophylla*, followed by *T. arjuna*, *P. pinnata*, *S. glauca*, *S. cumini*, and *M. elengi*. Carbon storage and sequestration potentials of various tree species were studied using a dynamic growth model (CO2FIX) and found that the sequestration efficiency of fast-growing trees is higher than that of slow-growing ones (Kaul *et al*., 2010). There are reports on the higher growth performances of *S. macrophylla* (Superales, 2016), *T. arjuna* (Maji *et al*., 2017), *P. pinnata* (Bohre *et al*., 2014), *S. glauca* (Anil, 2009). The higher uptake of CO_2 by *S. macrophylla, T. arjuna,* and *P. pinnata* in the present study can be attributed to the fast growth rate and higher biomass accumulation by the plants. Though *S. glauca* is considered a fast-growing species, their resultant release of Carbon dioxide during the night makes them a comparatively poor candidate for CO_2 sequestration.

Influence on microclimatic conditions

The microclimate surrounding a plant has a major effect on its growth and metabolism. In the growth chamber having varying influxes of CO_2, the temperature and humidity are likely to vary considerably, which in turn will influence the growth and metabolism of the plants contained within it. Along with this, the metabolic status of plants also influences the temperature and humidity associated with their systems.

In the present study, an attempt has been carried out to assess the changes in the microclimatic conditions brought about by the growth of six tree species maintained in growth chambers, and are subjected to varying levels of Carbon dioxide supply.

The temperature and day flux in CO_2 are shown to be negatively correlated in all the plants except *S. macrophylla*. *T. arjuna* showed a strong negative correlation

between the day flux of CO_2 and the temperature after CO_2 supplementation. The change in day temperature inside the TC with *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi, and S. cumini* is lower compared to that of the CC. Similarly, the night variation in temperature inside the TC with *T. arjuna*, *S. macrophylla*, *M. elengi, and S. cumini* is lower compared to that of the CC.

Similarly, CO_2 flux and humidity are found to be positively correlated in all the plants except *S. macrophylla*. In *S. macrophylla*, a negative correlation (-0.08) is observed between CO_2 flux and humidity. In *T. arjuna* (0.58), *P. pinnata* (0.22), *S. glauca* (0.4), *M. elengi* (0.29), and *S. cumini* (0.31), a positive correlation is observed.

The correlation between temperature and humidity is found to be negative in all the plants under elevated CO_2 conditions and is significant. A highly significant negative correlation is observed between temperature and humidity in *M. elengi* (-0.88***). A negative correlation (-0.66*) is found in *T. arjuna*, *S. macrophylla* (-0.78*), *P pinnata* (-0.61), *S. glauca* (-0.61*), *M. elengi* (-0.88***), and *S. cumini* (-0.6*).

Multiple factors are responsible for the reduction in temperature inside the growth chambers retained under elevated levels of CO_2. Carbon dioxide, being a greenhouse gas, absorbs solar radiation and normally gets heated up (Zhong and Haigh, 2013), especially when using growth chambers having a higher concentration of CO_2 (Schmidt *et al*., 2010). Collision of CO_2 molecules as a result of high pressure inside TC due to Carbon dioxide produces heat which can also be an explanation for the increased temperature in the chamber (Charriere *et al*., 2010). Despite this, a reduction in temperature was noticed in the TC, compared to the control. This can happen as a result of increased transpiration and resultant humidity attributed by the plants within the chambers, This was evident in the polyvinyl chloride walls of the chamber, which were noted to have a higher extent of water condensation throughout the study. However, the chamber containing *S. macrophylla* showed an increase in temperature and a decrease in humidity. The results are in line with the reports of Ahmed *et al*. (2007) on *S. macrophylla.* An increase in temperature can be a result of the increased level of CO_2, whereas a decrease in the level of humidity can be due to the lesser rate of transpiration by the plant, circumventing a stressful condition attributed to an increase in the levels of Carbon dioxide.

Upon consolidation of the present study, it is noted that out of the six tree species under study, CO_2 assimilation efficiency (net day flux) was higher in *S. macrophylla* followed by *T. arjuna, P. pinnata, S. glauca, S. cumini,* and *M. elengi*. Though the CO_2 assimilation efficiency of *S. glauca,* and *P. pinnata* are moderately high, their higher respiratory attribution under elevated levels of CO_2 are making them lesser performers in the pursuit of CO_2 sequestration. The lesser biomass production potential of *M. elengi* in a shorter duration is a hindrance to their selection for rapid sequestration efforts. The carbon assimilation potential of the above tree species thus provides valuable information for urban planners and policymakers for their continued efforts in carbon mitigation.

1.6 SUMMARY AND CONCLUSION

The present study in a broader perspective is an attempt to identify the tree species ideal for Carbon dioxide sequestration, especially for tropical climatic conditions. For this, an inventory of the tress species has been carried out through literature. Selected species were reared in the nursery for a specific period and were subjected to Carbon dioxide sequestration studies using controlled growth chambers.

The specific objectives outlined in the study include (1) identification of avenue tree species ideal for tropical climatic conditions and collection of information on their natural mode of multiplication and growth (2) multiplication in nurseries and maintenance up to desired stages of growth for experimentation along with standardization of growth conditions and acclimatization of characteristics (3) conduct of CO_2 sequestration studies using controlled growth chambers under varying concentrations of CO_2 and other growth conditions (4) assessment of the changes in microclimatic conditions associated with the chambers, which are brought about by the growth of plants under varying levels of CO_2 supply (5) assessment of the changes in growth and biomass contents of plants under varying levels of CO_2 supply (6) assessment of the biochemical and minerlogicl attributes associated with the plants under varying levels of CO_2 and (7) listing up of plants having higher CO_2 sequestration potential and optimization of conditions of highest sequestration efficiency.

Accordingly, six different tree species (*Terminalia arjuna* (Roxb. ex DC.), *Swietenia macrophylla* King, *Pongamia pinnata* (L.) Pierre, *Simarouba glauca* DC., *Mimusops elengi* L., and *Syzygium cumini* (L.) Skeels) were selected for the present study. The plantlets were reared and maintained in the polyhouse for acclimatization.

The experimentation was carried out in two chambers, each with a volume of 6.32 m^3, mended with PVC frames, and covered with 1 mm thick transparent polyvinyl chloride sheets. The control chamber (CC) was equipped with the facility for the supply of ambient air through an air compressor, whereas the treatment chamber (TC) was equipped with the facility for the supply of CO_2 – air mixture in specific doses. Both chambers were fitted with facilities for the analysis of CO_2 (ppm), temperature (°C), and humidity (%).

For experimentation, uniformly grown tree saplings (18 months old) were introduced to the CC and supplied daily with ambient air for 15 minutes in the morning and evening, maintaining a resultant CO_2 concentration of 475±42 ppm. Similarly, in the TC, the CO_2-air mixture was supplied daily in the morning and evening (15 minutes), maintaining a resultant CO_2 concentration of 979.83±30.93 ppm. The magnitude of CO_2 concentration (ppm) along with temperature (^oC) and humidity (%) within the chambers was monitored twice a day at 9 a.m. and 6 p.m.. Accordingly, the day and night flux in CO_2 associated with CC and TC were assessed.

Similarly, a standardization study was undertaken to assess the retention percentage and daily flux of gases associated with both chambers. For this, the entire experimentation was undertaken in the respective chambers without plants, and with a simultaneous supply of air/air - CO_2 mixture and subsequent analysis of temperature, humidity, and CO_2 at specific time intervals of the day (9 a.m. and 6 p.m.) for 15 days. Accordingly, the day and night flux in CO_2 associated with CC and TC in the absence of plants was monitored. From the results of the standardization study and experimentation using plants, the gross and net flux of CO_2 was calculated. The net flux was calculated as the difference in the CO_2 values obtained during experimentation using plants with that of the standardization results.

The results on the CO_2 assimilation potentials of the tree species are given in this Chapter (Chapter I). However, changes in the growth, and biochemical aspects of plants together with the changes in soil characteristics are depicted in Chapter II.

Considering various attributes, it has been noticed that the assimilation of Carbon dioxide was higher in *S. macrophylla*, followed by *T. arjuna*, *P. pinnata*, *S. glauca*, *S. cumini*, and *M. elengi*. From the gross value of night flux (TC), the attribution of Carbon dioxide to the system was higher with *S. glauca* followed by *P. pinnata*, *S. cumini*, *T. arjuna*, *M. elengi*, and *S. macrophylla*. Considering the relative performances of the plants under day and night fluxes, it has been noticed that *S. macrophylla*, followed by *T. arjuna*, are effective in carbon assimilation, with higher accumulation during daytime and lower releases during night. Species like *P. pinnata* and *S. glauca*, though significant in terms of daytime CO_2 assimilation, their resultant release during nighttime was higher, showing lesser performances in the perspective of Carbon sequestration. The lesser biomass production potential of *M. elengi* in a shorter duration is a hindrance to their selection for rapid sequestration efforts.

The temperature and day flux in CO_2 are shown to be negatively correlated in all the plants except *S. macrophylla*. The species *T. arjuna* showed a strong negative correlation between the day flux of CO_2 and the temperature after CO_2 supplementation. The change in day temperature inside the TC with *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi,* and *S. cumini* is lower compared to that of the CC. Similarly, the night variation in temperature inside the TC with *T. arjuna*, *S. macrophylla*, *M. elengi, and S. cumini* is lower compared to that of the CC. Similarly, CO_2 flux and humidity are found to be positively correlated in all the plants except *S. macrophylla*. The correlation between temperature and humidity is found to be negative in all the plants under elevated CO_2 conditions and is significant. All the correlations are statistically significant.

The Carbon dioxide assimilation potential of the above tree species thus provides valuable information for urban planners and policymakers for their continued efforts in carbon mitigation through carbon offset planting.

CHAPTER II

GROWTH AND BIOCHEMICAL RESPONSES OF SELECTED TREE SPECIES

2.1. INTRODUCTION

Trees are widely been recognized as the major sponges of carbon and significantly contribute to the decarbonization of the atmosphere (Saral *et al*., 2017). Trees effectively sequester carbon for an extended duration, with minimal release into the atmosphere. They do so by absorbing Carbon dioxide from the atmosphere and converting it to biomass through the process of photosynthesis. On average, carbon sequestered by an individual tree per year is estimated to be 70 Kg in the Amity University campus, Noidby by Sharma *et al*. (2020). Different authors have brought different rates for carbon sequestration which varies by the species and agroclimatic conditions.

The carbon sequestration capability of plant species depends on various attributes like growth (age, height, girth, size, canopy diameter, etc.), biomass accumulation rates, wood density, etc. (Sharma *et al*., 2020). Young plants accumulate biomass and sequester more atmospheric carbon (Anil, 2009) than old trees, which in turn release more carbon into the atmosphere via respiration, tree cutting, and decomposition (Nowak and Crane, 2002; Hai *et al*., 2015). In addition to the vegetation type, the soil composition, and the prevailing climatic conditions contribute to the rate of CO_2 sequestration (International Institute for Sustainable Development). Thus, planting trees is a common method of combating climate change, which is otherwise attributed to increased CO_2 levels in the atmosphere (Dubal *et al*., 2013).

As it is reported that the carbon assimilation potentials of trees vary with the species, the stage of growth, and other environmental conditions, an attempt has been carried out to assess the responses of six tree species namely *Terminalia arjuna* (Arjun), *Swietenia macrophylla* (Mahagony), *Pongamia pinnata* (Ungu), *Simarouba glauca* (Paradise tree), *Mimusops elengi* (Spanish cherry), and *Syzygium cumini* (Java plum) to elevated levels of CO_2 and the results on gasesous flux have been attempted in Chapter I. The present Chapter is an extension of the previous work and focuses

mainly on the growth and biochemical responses of the above species to elevated levels of Carbon dioxide. The objectives undertaken in the present Chapter are outlined below:

- Assessment of the growth and changes in plant biomass content under varying levels of Carbon dioxide supply.
- Assessment of the biochemical responses of the plants subjected to varying concentrations of Carbon dioxide.
- Listing up of plants having higher Carbon dioxide sequestration potential and optimization of conditions of highest sequestration efficiency.

The growth and biochemical responses of plants to varying conditions differ considerably. Following is a review in this direction.

2.2. REVIEW OF LITERATURE

Humanity relies heavily on the ecosystem services provided by tropical forests. Gardner *et al*. (2009) reported that these ecosystems support a minimum of two-thirds of the terrestrial biodiversity on Earth and are vital to biogeochemical cycles. An estimated 55 percent of the worldwide carbon stock is sequestered in forests (Pan *et al*., 2011). However, the magnitude of it varies considerably. The degree of tissue maturity influences the growth rate of trees. Also, deciduous species lack green photosynthetic leaves for a portion of the year, whereas evergreen trees possess the capacity to continuously absorb carbon (Lee and Jarvis,1995). Research studies have established that forests are among the most significant carbon sinks as trees store carbon for decades in their main stem wood, bark, branches, leaves, and roots (Nizami *et al*., 2014).

It is reported by many that immature plantations can store carbon in relatively larger quantities, whereas fully developed plantations function as reservoirs. In comparison to short-rotation species, long-rotation species such as teak (*Tectona grandis*) have longer carbon locking periods (Sreejesh *et al*., 2013). Roadside trees, whether they are cultivated or occur naturally, serve ecologically by sequestering carbon and aiding in the mitigation of climate change (Da Silva *et al*., 2012; Singh and Singh, 2015). Dubal *et al*. (2013) assert that *S. macrophylla* possesses a substantial capacity

to sequester atmospheric carbon, thereby contributing to the mitigation of the greenhouse effect.

Miria and Khan (2013) examined the carbon storage and annual growth rate of a variety of tree species. The research findings indicated that *Syzygium*, a species of ornamental and fruit-bearing tree, sequestered a greater quantity of carbon than *Pongamia*, a species of timber tree. In the study on the carbon storage capacity of *Eucalyptus* plantations, Du *et al*. (2015) reported that carbon sequestration was greatest in the subterranean regions compared to the aerial regions. This finding contrasts with the prevailing pattern in other plant species, wherein the above-ground portions exhibit a greater capacity for carbon sequestration.

The growth, biomass, carbon storage, and nutrient accumulation in *Gmelina* were reported by Swamy *et al*. (2003). A substantial surge in growth and biomass production was detected three years after planting, despite the species exhibiting modest levels of development and biomass production during the initial phase. The productivity of wasteland was enhanced by *Gmelina* plantations, which increased the availability of soil nutrients, particularly nitrogen and potassium. Stainback and Alavalapati (2002) investigated the carbon sequestration potential of rubber plantations and reported that with an increase in the age of the tree, the sequestration rate increased. The potential of *Swietenia macrophylla* in terms of aboveground biomass, CO_2 capture, and carbon storage was evaluated in a study conducted by Superales (2016). Additionally, the findings revealed that the wood samples accumulated an average biomass of 268.26 g and a greater quantity of CO_2 (16.244 g) than the leaves (12.111 g) and bark (5.934 g).

Kaul *et al*. (2010) estimated the carbon stock of Sal Forest as 82 Mg C/ha/yr. The net annual carbon sequestration rates were achieved for fast-growing short rotation Poplar (8 Mg $Cha^{-1}yr^{-1}$) and *Eucalyptus* (6 Mg $Cha^{-1}yr^{-1}$) plantations, followed by moderately growing Teak forests (2 Mg $Cha^{-1}yr^{-1}$) and slow growing long rotation Sal forests (1 Mg $Cha^{-1}yr^{-1}$). According to this study, fast-growing short-rotation plantations typically exhibit high net annual carbon fluxes over a brief period, which is relevant to the dynamic growth model (CO_2FIX) used in the study. Swamy *et al*. (2003) conducted an assessment of the growth, biomass, carbon storage, and nutrient distribution of *Gmelina arborea* Roxb. The carbon content of the tree components

was highest in the stem wood (56.25%), followed by the branches (19.8%) and the roots (18.51%). *Pongamia pinnata* exhibited an increase in net biomass production and carbon sequestration as the age of plantations increased, as reported by Bohre *et al*. (2014). The list of tree species concerning their carbon storage is depicted in **Table 2.2.1**.

Table 2.2.1. Tree species with their carbon storage potential.

Sl. No.	Tree Sp.	Family	Carbon storage	Reference
1	*Gmelina arborea* Roxb.ex Sm. (Gamhar)	Lamiaceae	31.37 Mg/ha or 31,370 kg/ha	Swamy *et al*., 2003
2	*Albizia lebbeck*	Fabaceae	33.85 ton/ha	Chavan and Rasal (2010)
3.	*Delonix regia*	Fabaceae	19.06 ton/ha	Chavan and Rasal (2010)
4.	*Shorea robusta* Gaertn. f	Dipterocarpaceae	82 Mg Cha^{-1}	Kaul *et al*., 2010
5.	*Populus deltoides* Marsh	Salicaceae	8 Mg $Cha^{-1}yr^{-1}$	Kaul *et al*., 2010
6.	*Eucalyptus tereticornis* Sm	Myrtaceae	6 Mg $Cha^{-1}yr^{-1}$	Kaul *et al*., 2010
7.	*Tectona grandis Linn. f*	Lamiaceae	2 Mg $Cha^{-1}yr^{-1}$	Kaul *et al*., 2010

2.2.1 Photosynthetic responses of tree species to elevated CO_2

Downregulation of photosynthesis in trees has been noted following extended periods of exposure to high levels of Carbon dioxide (Lewis *et al*., 1996; Atkinson *et al*., 1997; Rey and Jarvis, 1998). This phenomenon is frequently ascribed to an inequitable source-sink relationship resulting from the buildup of leaf carbohydrates (Webber *et al*., 1994; Van Oosten *et al*., 1994; Jones *et al*., 1982; Cheng *et al*., 1998).

Rasineni *et al.* (2013) reported an enhancement in the photosynthesis and productivity in *Gmelina arborea* due to elevated atmospheric CO_2. In response to elevated CO_2 levels, plants increase photosynthesis and decrease stomatal conductance. Photosynthesis ought to be stimulated by the exponential increase in atmospheric CO_2 as a result of enhanced Rubisco carboxylation. Research by Ceuleman *et al*. (1997) revealed that when rapidly growing tree species, such as

poplars, are exposed to elevated CO_2 for 17 months, their Rubisco activity decreases. Increased CO_2 levels stimulated net photosynthesis in Poplars grown in OTC (Open Top Chambers) for four months. Prolonged exposure to increased Carbon dioxide levels resulted in alterations in the biochemical, morphological, and physiological parameters, which subsequently nullified the initial stimulus for photosynthesis (Makino and Mae, 1999). A frequent correlation has been observed between stomatal conductance and net photosynthesis (Salisbury and Ross, 1992). In general, the closure of stomata results in a reduction in net photosynthetic rates (Heber *et al*. 1986, Bunce 1988). Therefore, alterations in photosynthetic rates may result from modifications in stomatal conductance (Meng and Arp 1993). Many studies have put forward the hypothesis that increased concentrations of Carbon dioxide may enhance the efficiency of plant photosynthesis and water use efficiency (WUE) (Drutaa, 2001). Yasaki *et al*. (2004) observed that the NET assimilation in *Larix kaempferi* showed an increase over 107 days when exposed to elevated CO_2 levels. The reduction in photosynthesis activity observed in the presence of high CO_2 levels is ascribed to diminished stomatal conductance, as evidenced by reduced stomatal area and density (Zheng *et al*., 2019). In an Open-Top- Chamber investigation conducted by Moutinho Pereira *et al*. (2009), changes in stomatal conductance and an increase in photosynthesis were observed in *Vitis vinifera*. *Betula pendula* exhibited a 30% increase in photosynthetic rate when subjected to escalating CO_2 levels in an open-top chamber for three years (Riikonen *et al*., 2005). Several tree species, including *Salix dasyclados*, *Castanea sativa*, *Cedrus atlantica*, *Citrus sinensis*, *Garcinia mangostana*, *Picea mariana*, *Pinus ponderosa*, and *Populus* hybrids, exhibit downregulation of photosynthesis in response to elevated CO_2 levels (Ceulemans and Mousseau, 1994). An assortment of scientists employed Open-Top-Chamber experiments to determine the photosynthetic responses of *Brassica* sp., *Medicago sativa*, *Betula pendula*, *Prunus avium*, *Cicer arietinum*, *Vitis vinifera*, *Hymenaea courbaril*, *Solanum tuberosum*, *Azadirachta indica*, *Melia dubai*, *Prunus persica*, *Arachis hypogea*, *Raphanus sativus*, and *Fragaria ananassa*. The duration of Carbon dioxide (CO_2) exposure to plants varied between 10 days and 4.5 years. The alterations in the photosynthetic reactions of particular species in response to increased CO_2 levels are detailed below in **Table 2.2.2**

Table 2.2.2. Photosynthetic response of tree species under elevated CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	Photosynthetic response	Reference
1.	*Fagus sylvatica* (Beach)	Fagaceae	2 years	Varies slightly depending on the season	Epron *et al*., 1996
2	*Raphanus sativus*	Brassicaceae	46 days	+28%	Usuda and Shimogawara (1998)
3	*Glycine max*	Fabaceae	Meta-analysis	+59%	Ainsworth *et al*., 2002
4	*Arachis hypogea*	Fabaceaae	2 weeks	+42.8%	Vu (2005)
5	*Betula pendula*	Betulaceae	3 years	+30%	Riikonen *et al*., 2005
7	*Solanum tuberosum*	Solanaceae	5 weeks	+10 to 40%	Katny *et al*., 2005
8	*Cucumis sativus*	Cucurbitaceae	14 days	Increases	Aguera *et al*., 2006
9	*Raphanus sativus*	Brassicaceae	10 days	Increases	Urbonaviciute *et al*., 2006
10	*Daucus carota*	Apiaceae	30 days	+75.9%	Thiagarajan and Lada (2007)
11	*Vitis vinifera*	Vitaceae	2 years	+15%	Moutinho Pereira *et al*., 2009
12	*Azadirachta indica, Melia dubai*	Meliaceae	125 days	+110%	Janani *et al*., 2016
13	*Santalum album*	Santalaceae	120 days	+211.5%	Lamani (2016)
14	*Theobroma cacao*	Malvaceae	13 weeks	+105%	Lahive *et al*., 2018
15	*Quercus mangolica*	Fagaceae	60 days	Increases	Wang *et al*., 2019

[+ sign indicates percentage increase over the control]

2.2.2 Elevated CO_2 and biomass responses

According to a study by Rey and Jarvis (1997), increased CO_2 levels in *Betula pendula* led to a 58% increase in biomass. Experimental studies utilizing Free Air CO_2 enrichment (FACE) on *Populus tremuloids*, *Acer saccharum*, and *Betula papirifera* revealed that these plants exhibited an increase in biomass production when exposed to elevated concentrations of CO_2 (Kallarackal and Roby, 2012). Higher dry mass (55%) was accumulated by pine trees grown in environments with elevated CO_2 than by trees grown in ambient conditions (Jach and Cuelemans, 2000). Bohre *et al*. (2014) identified a positive correlation between the age of *Pongamia pinnata* plantations and their net biomass production and carbon

sequestration. Elevated photosynthetic rates were observed in *Prunus avium* (Centritto *et al*., 1999), *Fragaria ananassa* (Bunce, 2001), *Poplars* (Bernacchi *et al*., 2003), *Fagus sylvatica* (Lotfiomran *et al*., 2016), and Spinach (Jain *et al*., 2007), all of which exhibited a positive correlation with changes in biomass production. A study by Superales (2016) revealed that in contrast to the leaves and bark, saplings of *Swietenia macrophylla* absorbed a greater quantity of CO_2 in their wood. According to Gunn *et al*. (1999), the plants *Dactylis glomerata*, *Bellis perennis*, and *Trifolium repens* exhibited a higher dry mass (total) at 700 ppm of CO_2 than at 350 ppm. The leaf area ratios of all plants maintained in an elevated Carbon dioxide environment were found to be reduced. A 60-day open-top chamber study was conducted by Hari Haran *et al*. (2015) on *Eucalyptus tereticornis*, during which an increase in leaf thickness, fresh weight, and dry weight was observed. Changes in biomass under elevated levels of Carbon dioxide in selected plant species are listed in **table 2.2.3**.

Table 2.2.3. Changes in biomass of plants under elevated CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	Biomass (%)	Reference
1	*Castanea sativa*	Fagaceae	7 months	Total dry mass (-42.6%)	Mousseau and Enoch (1989)
2.	*Fagus sylvatica*	Fagaceae	2 years	Leaf dry mass (+67.56 %)	Epron *et al*., 1996
3	*Raphanus sativus*	Brassicaceae	46 days	Dry matter (+111%)	Usuda and Shimogawara (1998)
4	*Solanum lycopersicum*	Solanaceae	46 days	Total biomass (+8.69%)	Juan *et al*., 2007
5	*Quercus mongolica, Kalopanax septemlobus, Betula maximowicziana, Acer mono*	Fagaceae, Araliaceae, Betulaceae, Aceraceae	2 years	Biomass increased	Watanabe *et al*., 2010
6	*Carthamus tinctorius*	Asteraceae	6 months	Total above-ground dry weight (+51%)	Mohamed *et al*., 2013

7	*Azadirachta indica, Melia dubai*	Meliaceae	125 days	Shoot biomass increased	Janani *et al*., 2016
8	*Elaeis guineesis*	Aracaceae	3 months	(+122.4%)	Ibrahim *et al*., 2018
9	*Theobroma cacao*	Malvaceae	13 weeks	Leaf dry mass (+28.5%)	Lahive *et al*., 2018

[+ sign indicates percentage increase over the control, - sign indicates percentage decrease over the control]

2.2.3 Elevated CO_2 and stomatal conductance

Stomatal conductance is the rate of CO_2 entering or water vapor exiting through the stomata. The leaf exerts direct biological control over it via guard cells that encircle the stomatal pore (Taiz and Zeiger,1991). Stomata, which are minute apertures situated at the upper and lower surfaces (mostly) of a leaf, function to absorb Carbon dioxide (CO_2) and release water vapour. Stomatal conductance determines the turgor pressure and osmotic potential of guard cells in a direct manner. It is also influenced by the dimensions of the stomata, including their aperture, density, and size. A frequent correlation has been observed between stomatal conductance and net photosynthesis (Salisbury and Ross, 1992). In general, the closure of stomata resulted in a reduction in net photosynthetic rates (Heber *et al*., 1986; Bunce, 1988). Therefore, alterations in photosynthetic rates may result from modifications in stomatal conductance (Meng and Arp, 1993).

According to Haworth *et al*. (2016), impaired stomatal control was associated with reduced photosynthetic physiology in crop species grown at elevated CO_2. The regulation of stomatal conductance by plants enables them to maintain a balance between CO_2 absorption for photosynthesis and water loss, thereby optimizing water use efficiency. An elevation in the atmospheric concentration of Carbon dioxide (CO_2) induces an increase in photosynthesis and a decrease in stomatal conductance in numerous plant species, thereby augmenting carbon uptake and diminishing water loss. Photosystem II's performance was unaffected by growth at elevated CO_2, indicating that high CO_2 levels had no detrimental effect on photosynthetic physiology. Photosynthetic regulation of stomatal conductance is directly impaired at elevated CO_2 levels.

Likewise, an investigation was conducted on the stomatal responses and net photosynthesis of Mongolian Oak for 210 days under conditions of elevated CO_2 concentrations of 80 and 700 ppm. In this study, enriched CO_2 increased the photosynthetic rate while decreasing stomatal density (Wang *et al*., 2019). The meta-analysis study conducted by Huang and Xu (2015) documented enhanced water use efficiency and photosynthesis, in addition to a decrease in transpiration. A rise in the Carbon dioxide concentration in the atmosphere induced an upregulation of photosynthesis and a downregulation of stomatal conductance in numerous plant species, thereby augmenting carbon uptake and diminishing water loss. Vu (2005) observed that *Arachis hypogaea* exhibited a decline in stomatal conductance and transpiration, but an increase in water use efficiency, in their elevated CO_2 study. The changes in the stomatal conductance in plants under elevated levels of CO_2 are depicted in **table 2.2.4**.

Table 2.2.4. Changes in stomatal conductance of plants under elevated CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	Stomatal characteristics	Reference
1.	*Castanea sativa*	Fagaceae	7 months	gs (-4%)	Mousseau and Enoch (1989)
2	*Glycine max*	Fabaceae	Meta-analysis	gs (-40%)	Ainsworth *et al*., 2002
3.	*Cucumis sativus*	Cucurbitaceae	14 days	I (+15.7%)	Aguera *et al*., 2006
4	*Daucus carota*	Apiaceae	30 days	I (-9.52%)	Thiagarajan and Lada (2007)
5	*Vitis vinifera*	Vitaceae	2 years	gs (+56.1%)	Moutinho Pereira *et al*., 2009
6	*Carthamus tinctorius*	Asteraceae	6 months	gs (-29%)	Mohamed *et al*., 2013
7	*Santalum album*	Santalaceae	120 days	gs (+50%)	Lamani (2016)
8	*Prunus persica*	Rosaceae	38 days	gs (+37.93%)	Davidson *et al*., 2016
9.	*Theobroma cacao*	Malvaceae	13 weeks	I (+10.07%)	Lahive *et al*., 2018
10.	*Quercus mangolica*	Fagaceae	60 days	I (+0.53%)	Wang *et al*., 2019
11	*Glycine max*	Fabaceae	27 days	Stomatal area-(+13.82%)	Zheng *et al*., 2019

[+ sign indicates percentage increase over the control, - sign indicates percentage decrease over the control] [I indicate stomatal index, gs indicates stomatal conductance]

2.2.4 The changes in water use efficiency and transpiration under elevated levels of Carbon dioxide

Vu (2005) observed that transpiration and stomatal conductance decreased in *Arachis hypogaea* (Peanut) during an elevated CO_2 study, whereas water use efficiency increased. Mohamed *et al*. (2013) conducted an experiment in which they observed a 29% and 18% decrease in stomatal conductance and transpiration, respectively, in *Carthamus tinctorius* subjected to elevated CO_2. Water use efficiency in *Theobroma cacao* and *Daucus carota* was found to be enhanced in the presence of elevated CO_2 levels, as indicated by reduced stomatal conductance (Lahive *et al*., 2018; Thiagarajan and Lada, 2007). Reduced stomatal conductance may have contributed to a decline in transpiration in *Cucumis sativus* and *Daucus carota* when subjected to elevated CO_2 concentrations (Aguera *et al*., 2006; Thiagarajan and Lada, 2007). In contrast to the aforementioned findings, the results of Moutinho Pereira *et al*. (2009) and Lamani (2016) indicate that transpiration of *Santalum album* and *Vitis vinifera* increased in the presence of elevated CO_2. Changes in water use efficiency (WUE)/ and transpiration (E) of plants under elevated levels of Carbon dioxide are listed in **table 2.2.5**.

Table 2.2.5. Changes in water use efficiency of plants under elevated CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	WUE/ E	Reference
1	*Arachis hypogea*	Fabaceae	2 weeks	E (-12.5%) WUE (+55.5%)	Vu (2005)
2	*Cucumis sativus*	Cucurbitaceae	14 days	E (-6.38%)	Aguera *et al*., 2006
3	*Daucus carota*	Apiaceae	30 days	WUE(+112%) E (-3.125%)	Thiagarajan and Lada. (2007)
4	*Vitis vinifera*	Vitaceae	2 years	E (+39.97%)	Moutinho Pereira *et al*., 2009
5	*Carthamus tinctorius*	Asteraceae	6 months	E (-18%)	Mohamed *et al*., 2013
6	*Santalum album*	Santalaceae	120 days	E (+27.7%)	Lamani (2016)
7	*Theobroma cacao*	Malvaceae	13 weeks	WUE (+44%)	Lahive *et al*., 2018

[+ sign indicates percentage increase over the control, - sign indicates percentage decrease over the control] [WUE indicates water use efficiency, E indicates transpiration]

2.2.5 Elevated CO_2 and growth response of plants

The response of plant growth to increased CO_2 levels has been the subject of investigation by numerous researchers. Elevated Carbon dioxide levels, in general, have been associated with increased photosynthetic activity and glucose availability, promoting cellular development, and encompassing expansion and division (Huang and Xu, 2015). The study of Lamani (2016) documented a favourable outcome in *Santalum album*, wherein the seedlings exhibited an increase in height as a result of enhanced carbon assimilation via CO_2. Four hardwood species, including two ring-porous species (*Quercus mongolica* and *Kalopanax septemlobus*) and two diffuse-porous species (*Betula maximowicziana* and *Acer mono*), were exposed to enriched CO_2 for two years in FACE at a concentration of 500 ppm and an ambient CO_2 concentration of 370 ppm. The trees' height, stem diameter, and biomass increased in response to the enriched CO_2. Species-specific anatomical variations were observed in the wood of trees exposed to elevated CO_2 (Watanabe *et al*., 2010). Saha *et al*. (2013) worked on *Cicer aerietinum* using open-top chamber studies and found that elevated CO_2 conditions led to an increase in plant height and leaf area index. Extensive research has been conducted to determine the carbon sequestration capacity of various plantations in Kerala, including *Mangifera indica*, *Cocos nucifera*, *Swietenia macrophylla*, *Tectona grandis*, *Gmelina arborea*, *Pongamia pinnata*, and *Tectona grandis* (Superales, 2016; Bohre *et al*., 2014; Miria & Khan, 2013; Sreejesh *et al*., 2013). Both Ruiz-Vera *et al*. (2021) and Ainsworth *et al*. (2002) documented a growth spurt in the height of *Glycine max* and cassava plants, respectively. Lahive *et al*. (2018) and Pal *et al*. (2004) both documented a rise in stem thickness in *Trifolium alexandrium* and *Theobroma cacao*. Previous studies have documented an augmentation in leaf area in response to increased Carbon dioxide levels (Campbell *et al*., 2001; Sanz-Saez *et al*., 2010; Mohamed *et al*., 2013; Lahive *et al*., 2018; Ruiz-vera *et al*., 2021). When *Fagus sylvatica* was exposed to enriched CO_2, both the palisade and spongy parenchyma of the leaves grew in thickness. Elevated leaf thickness is indicated by a greater leaf mass per unit area (Epron *et al*., 1996). In a similar vein, research conducted in growth chambers on tomato seedlings revealed that exposure to CO_2 increased both stem thickness and plant height (Juan *et al*., 2007). Chakraborty *et al*. (2015) documented that Brassica species that were subjected to elevated CO_2 levels within an open-top chamber exhibited enhanced photosynthetic rate, leaf area, and growth. Elevated CO_2

increased the height and leaf area index of *Clidemia hirta* and *Melastoma malabathricum*. The CO_2 concentration of the control was 400 ppm, while that of the treatment was 800 ppm (Wan Nur and Wan Juliana, 2017). The morphological responses of plants under elevated levels of CO_2 are listed in **table 2.2.6**.

Table 2.2.6. Growth and morphological response of plants under elevated CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	Morphological character	Reference
1	*Castanea sativa*	Fagaceae	7 months	Leaf area (-24.82%)	Mousseau and Enoch (1989)
2	*Fagus sylvatica*	Fagaceae	2 years	Leaf area (+53.2 %)	Epron *et al*., 1996
3	*Quercus suber*	Fagaceae	14 months	Leaf thickness (3.09%)	Faria *et al*., 1996
4	*Betula pendula*	Betulaceae	4.5 years	Tree height (+5.82%) Leaf area (+42.25%)	Rey and Jarvis, (1998)
5	*Glycine max*	Fabaceae	Meta-analysis	Leaf area (+11%)	Ainsworth *et al*., 2002
6	*Arachis hypogea*	Fabaceae	2 weeks	Leaf area (+7.84%)	Vu (2005)
7	*Betula pendula*	Betulaceae	3 years	Leaf thickness (-2.65%)	Oksanen *et al*., 2005
8	*Arabidopsis thaliana*	Brassicaceae	-	Leaf thickness (+5.3%)	Teng *et al*., 2006
9	*Cucumis sativus*	Cucurbitaceae	14 days	LAI (+139.5%)	Aguera *et al*., 2006
10	Tomato seedling	Solanaceae	46 days	Stem thickness (-13.51%)	Juan *et al*., 2007
11	*Vitis vinifera*	Vitaceae	2 years	Leaf thickness (+4.47%)	Moutinho Pereira *et al*., 2009
12	*Quercus mongolica, Kalopanax septemlobus, Betula maximowicziana, Acer mono*	Fagaceae, Araliaceae, Betulaceae, Sapindaceae	2 years	Plant height, Stem diameter- Increased	Watanabe *et al*., 2010
13	*Carthamus tinctorius*	Asteraceae	6 months	Leaf area index (+28%)	Mohamed *et al*., 2013

14	*Prunus persica*	Rosaceae	38 days	Plant height (-4%), Leaf area (-9.93%)	Davidson *et al*., 2016
15	*Elaeis guineesis*	Aracaceae	3 months	Leaf area (+35.8%) Plant height (+52.3%)	Ibrahim *et al*., 2018
16	*Theobroma cacao*	Malvaceae	13 weeks	Leaf area (+18.7%)	Lahive *et al*., 2018
17	*Glycine max*	Fabaceae	27 days	Leaf thickness (+17.39%)	Zheng *et al*., 2019

[+ sign indicates percentage increase over the control, - sign indicates percentage decrease over the control] [LAI indicates Leaf area index]

2.2.6 Elevated CO_2 and biochemical responses of plants

In response to increased levels of CO_2, plants undergo varied physiological adjustments, that result in altered biochemical synthesis. Various researchers, including Delucia *et al*. (1985), Faria *et al*. (1996), Nakano *et al*. (1997), Al-Rawahy *et al*. (2013), Jeong *et al*. (2018), Kumari *et al*. (2019), and Loladze *et al*. (2019), have investigated the effects of elevated CO_2 on plant pigmentation. Saha *et al*. (2013) undertook studies on *Cicer aerietinum* using an open-top chamber and reported that elevated CO_2 conditions led to an increase in chlorophyll content and a decrease in carotenoids. Hariharan *et al*. (2015) observed an increase in chlorophyll and carotenoids in *Eucalyptus tereticornis* under elevated CO_2 conditions during 60 days of research using open-top chambers. A seven-month exposure of *Castanea sativa* to an enriched CO_2 concentration of 700 ppm, in response to an ambient concentration of 350 ppm in the growth chamber resulted in a decrease in chlorophyll content (Mousseau and Enoch, 1989). *Capsicum annuum* exhibited a reduction in chlorophyll content when exposed to CO_2 enrichment (Kumari *et al*., 2019). Several researchers, including Hendrix *et al*. (1994), Faria *et al*. (1996), Kuetgen and Chen (2001), Ainsworth *et al*. (2002), Urbonaviciute *et al*. (2006), Aguera *et al*. (2006), and Ibrahim *et al*. (2018), examined the effects of elevated CO_2 on the carbohydrate content of plants. An increase in foliar starch and other carbohydrates may impede the rate of CO_2 assimilation, potentially leading to alterations in the acclimatory responses and photosynthetic rates of C3 plants grown in environments with high CO_2 concentrations (Reddy *et al*., 2010). Several studies have documented alterations in the protein content of plants when exposed to CO_2 enrichment, including those by Ainsworth *et al*. (2002), Korner *et al*. (2005), Taub

et al. (2008), Sreenivasulu *et al*. (2015), Janani *et al*. (2016), and Dong *et al*. (2018). The phenol content exhibited variations in response to elevated CO_2, as documented by Bryant *et al*. (1983), Tognetti *et al*. (1999), and Ghasemzadeh *et al*. (2010). According to Tognetti *et al*. (1999), increased CO_2 had no significant effect on the growth or phenolic content of *Quercus virginiana*, but did increase the concentration of total non-structural carbohydrates. The 125-day experiment conducted by Janani *et al*. (2016) revealed a reduction in metabolite concentrations, including protein and phenol, in the presence of elevated CO_2 levels. When exposed to CO_2 concentrations ranging from 400 to 800 ppm, *Zingiber officinale* in an open-top chamber showed an increase in flavonoid and phenol content (Ghazemzadeh *et al*., 2010). Coley *et al*. (2002) found that increased CO_2 levels led to increases in leaf phenolic contents, starch, total non-structural carbohydrates, and C/N ratios, but had no effect on biomass accumulation. The treated chambers contained approximately 400 ppm higher CO_2 than the ambient. The changes in metabolites of plants under elevated CO_2 are listed in **table 2.2.7.**

Table 2.2.7. Changes in plant metabolites under elevated levels of CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	Metabolite	Reference
1	*Castanea sativa*	Fagaceae	7 months	Total chl (-32.47%)	Mousseau and Enoch (1989)
2	*Quercus suber*	Fagaceae	14 months	Starch(-139.7%) Chlorophyll (-5.01%) Protein (+25.86%)	Faria *et al*., 1996
3	*Fagus sylvatica*	Fagaceae	2 years	Reduction in chlorophyll	Epron *et al*., 1996
4	Tropical plants	-	6 months	Phenol (+48%) Starch Increased	Coley *et al*., 2002
5	*Arachis hypogea*	Fabaceae	2 weeks	Protein (-14.66%)	Vu (2005)
6	*Solanum tuberosum*	Solanaceae	5 weeks	Starch (+260.52%)	Katny *et al*., 2005
7	*Arabidopsis thaliana*	Brassicaceae	-	Starch (+78.7%)	Teng *et al*., 2006
8	*Cucumis sativus*	Cucurbitaceae	14 days	Starch (+691.3%)	Aguera *et al*., 2006
9	*Raphanus sativus*	Brassicaceae	10 days	Starch, Chlorophyll increases	Urbonaviciute *et al*., 2006
10	*Zingiber officinale*	Zingiberaceae	-	Phenol, flavonoids increased	Ghasemzadeh *et al*., 2010

11	*Azadirachta indica, Melia dubai*	Meliaceae	125 days	Protein, Phenol decreased	Janani *et al*., 2016
12	*Prunus persica*	Rosaceae	38 days	Starch (+60%)	Davidson *et al*., 2016
13	*Theobroma cacao*	Malvaceae	13 weeks	Nitrogen (-11.3%)	Lahive *et al*., 2018
14	Rare and endangered trees	-	3 years	Chlorophyll Increased	Jeong *et al*., 2018
15	*Glycine max*	Fabaceae	27 days	Starch (-20.13%)	Zheng *et al*., 2019

[+ sign indicates percentage increase over the control, - sign indicates percentage decrease over the control]

2.2.7 Elevated CO_2 on plant minerals and nutrients

There was frequently a positive correlation observed between the accumulation of carbohydrates resulting from increased photosynthetic activity and the rise in leaf carbon content induced by rising CO_2 concentration and temperature (Tjoelker *et al*., 1999). According to Guo *et al*. (2021) and Reddy and Zhao (2005), mineral concentrations, of calcium, magnesium, potassium, and sodium varied in the presence of CO_2 enrichment. Huluka *et al*. (1994), Keutgen and Chen (2001), Teng *et al*. (2006), Duval *et al*. (2012), and Ibrahim *et al*. (2018) have studied variations in sodium and potassium in different plant species under elevated CO_2 conditions. The fluctuations in the C/N ratio in plant tissues were investigated by Gifford *et al*. (2000). Kumari *et al*. (2019) in an open-field investigation on *Capsicum annuum* reported a decrease in the concentration of nutrients like nitrogen, magnesium, and potassium, along with an increase in calcium under conditions of enriched CO_2. Moutinho Pereira *et al*. (2009) revealed that with an increase in CO_2 levels in the open-top chamber, magnesium and carbon concentrations in *Vitis vinifera* also increased. Huluka *et al*. (1994) conducted a FACE experiment spanning five months and revealed that the concentration of mineral nutrients in *Gossypium hirsutum* decreased in the presence of elevated CO_2 (ambient: 370 ppm, elevated: 550 ppm). This study undertaken in FACE revealed a reduction in nitrogen concentration and an increase in the C/N ratio in plants. In an experiment undertaken by Dong *et al*. (2018), *Cucumis sativus* was subjected to three distinct concentrations of CO_2 (400, 625, and 1200 ppm) in an open-top chamber. Elevated CO_2 resulted in an elevated level of magnesium and calcium, but a sodium reduction. The mineral content of

herbaceous and woody plants was studied by Overdieck (1993). Under enriched CO_2, mineral content such as nitrogen, calcium, and magnesium decreased in *Fagus sylvatica* and *Aceretium pseudo-platanus*. Under conditions of increased CO_2, the concentration of potassium decreased in *Acer* and increased in *Fagus*.

The nutrient quality and biomass production of lettuce were found to increase when exposed to CO_2 concentrations of 400 and 700 ppm for 35 days in a growth chamber using two lettuce varieties with distinct pigments (Perez Lopez *et al*., 2015). A study conducted by Oksanen *et al*. (2005) observed that *Betula pendula* cultivated in a cylindrical open-top chamber with elevated CO_2 for three years causes a decrease in nitrogen concentrations and potassium levels. Nutrients including nitrogen, calcium, potassium, and magnesium exhibited a reduction when cultivated within chambers with an automatically controlled environment and increased CO_2. The changes in minerals and nutrients under elevated CO_2 are depicted in **table 2.2.8**.

Table 2.2.8. Changes in minerals and nutrients of plants under elevated CO_2

Sl No.	Plant sp.	Family	Duration of CO_2 treatment	Minerals and nutrients	Reference
1	*Gossypium hirsutum*	Malvaceae	5 months	Nitrogen (- 32.05%) Calcium (- 32.03%) Potassium(-22.88%)	Huluka *et al*., 1994
2	*Betula pendula*	Betulaceae	4.5 years	Nitrogen (+63.9%) Potassium (+22.47%) Calcium (+86.95%) Magnesium (+75%)	Rey and Jarvis (1997)
3	Strawberry	Rosaceae	3 weeks	Nitrogen (-42.7%) Potassium (- 41.5%) Magnesium(+13.63%)	Keutgen *et al*., 1997
4	Tropical plants		6 months	Carbon increased	Coley *et al*., 2002
5	*Betula pendula*	Betulaceae	3 years	Leaf thickness (-2.65%) Nitrogen (-14.97%) Potassium(- 13.54%) Calcium(-12.97%) Magnesium (- 8.81%)	Oksanen *et al*., 2005
6	*Arabidopsis thaliana*	Brassicaceae	-	Carbon (+10%) Nitrogen (-11.2%) Magnesium (-14.8%)	Teng *et al*., 2006
7	Tomato seedling	Solanaceae	46 days	C/N ratio (-9.86%)	Juan *et al*., 2007
8	*Vitis vinifera*	Vitaceae	2 years	Carbon (-2.01%) Nitrogen (+9.28%) Magnesium(+96.01%) Calcium (-11.45%)	Moutinho Pereira *et al*., 2009

9	*Elaeis guinensis*	Aracaceae	3 months	Calcium (-90.62%) Magnesium(-58.06%) Potassium(-36.36%)	Ibrahim *et al*., 2018
10	Woody plants		3 years	Nitrogen (-25.2%)	Jeong *et al*., 2018
11	Rare and endangered trees		3 years	C/N ratio decreased	Jeong *et al*., 2018
12	*Cucumis sativus*	Cucurbitaceae	3 months	Calcium, Magnesium- Increased Sodium- decreased	Dong *et al*., 2018
13	*Glycine max*	Fabaceae	27 days	Carbon (-3.23%) Nitrogen (+4.74%)	Zheng *et al*., 2019

[+ sign indicates percentage increase over the control, - sign indicates percentage decrease over the control]

2.2.8 Elevated CO_2 and soil characteristics

Numerous researchers have examined the effects of elevated CO_2 on the soil properties (soil carbon, pH, moisture, etc.) having the growth of different tree species (Korner *et al*., 2005; Jastrow *et al*., 2005; Rogers *et al*., 1999, Nelson *et al*., 2004; Dermody *et al*., 2007).

Increased CO_2 levels in *Triticum aestivum* resulted in a reduction in soil pH and carbon (Biose *et al*., 2016). Yuhui *et al*. (2017) investigated the responses of soil carbon and enzyme activity in *Glycine max* to elevated CO_2 levels. In a FACE experiment conducted by Kumeleh *et al*. (2009), where ambient CO_2 is 350 ppm and elevated CO_2 is 570 ppm, soil carbon increased in the presence of elevated CO_2. Marhan *et al*. (2010) conducted a five-year experiment in a wheat agroecosystem under elevated CO_2 conditions. The study unveiled noteworthy findings regarding the soil properties that change under such conditions. Elevated CO_2 levels were found to accelerate the decomposition of soil organic matter, which results in increased soil moisture. Increased CO_2 levels have an indirect impact on soil moisture, which in turn expedites the depletion of soil organic carbon. Experiments on CO_2 enrichments (ambient CO_2-360 ppm, elevated CO_2-720 ppm) were carried out in the semi-arid short grass of Colorado. The findings of these experiments indicated that the application of enriched CO_2 increased soil moisture content (Nelson *et al*., 2004). The biota and soil structure of nutrient-deficient grasslands were altered by six years of CO_2 enrichment experiments (Niklaus *et al*., 2003).

In this circumstance, the present study has been undertaken to assess the growth and biochemical responses of six tree species namely *Terminalia arjuna* (Combretaceae), *Swietenia macrophylla* (Meliaceae), *Pongamia pinnata* (Fabaceae), *Simarouba glauca* (Simaroubaceae), *Mimusops elengi* (Sapotaceae), and *Syzygium cumini* (Myrtaceae) to elevated levels of CO_2.

2.3. MATERIALS AND METHODS

The design of the Carbon dioxide-controlled chambers and associated facilities together with the nursery trials related to the multiplication of plants and maintenance up to desired stages of growth for experimentation along with standardization of growth conditions and acclimatization of characteristics are presented in Chapter I.

Standardization studies concerning the growth chambers and the conduct of sequestration studies using selected plants under varying concentrations of Carbon dioxide along with an assessment of the changes in microclimatic conditions associated with the chamber brought about by the growth of plants are also discussed in Chapter I.
In this chapter, attempts have been carried out to assess the growth and biochemical responses of plants subjected to experimentations under varying levels of CO_2. This chapter focuses on studying the growth, metabolites, minerals, nutrients of plants, FTIR analysis of plants and soil characteristics under elevated CO_2 conditions.

The growth measurements (shoot) and biochemical estimations of plants were undertaken on days 1, 5, 10, and 15. The root measurements were undertaken on days 1 and 15. The growth parameters analyzed include plant height, stem diameter, leaf length, leaf breadth, leaf number, leaf area, moisture content, and plant biomass. The biochemical parameters analyzed include pigments (chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid), metabolites (carbohydrate, protein, phenol), minerals (calcium, magnesium, sodium, potassium, copper, zinc, and nutrients (carbon, nitrogen). The soil characteristics studied include moisture, pH, carbon, nitrogen, phosphorus, and potassium. The experimental outline is given in **figure 2.3.1** and the experimental studies using tree species in **figure 2.3.2**.

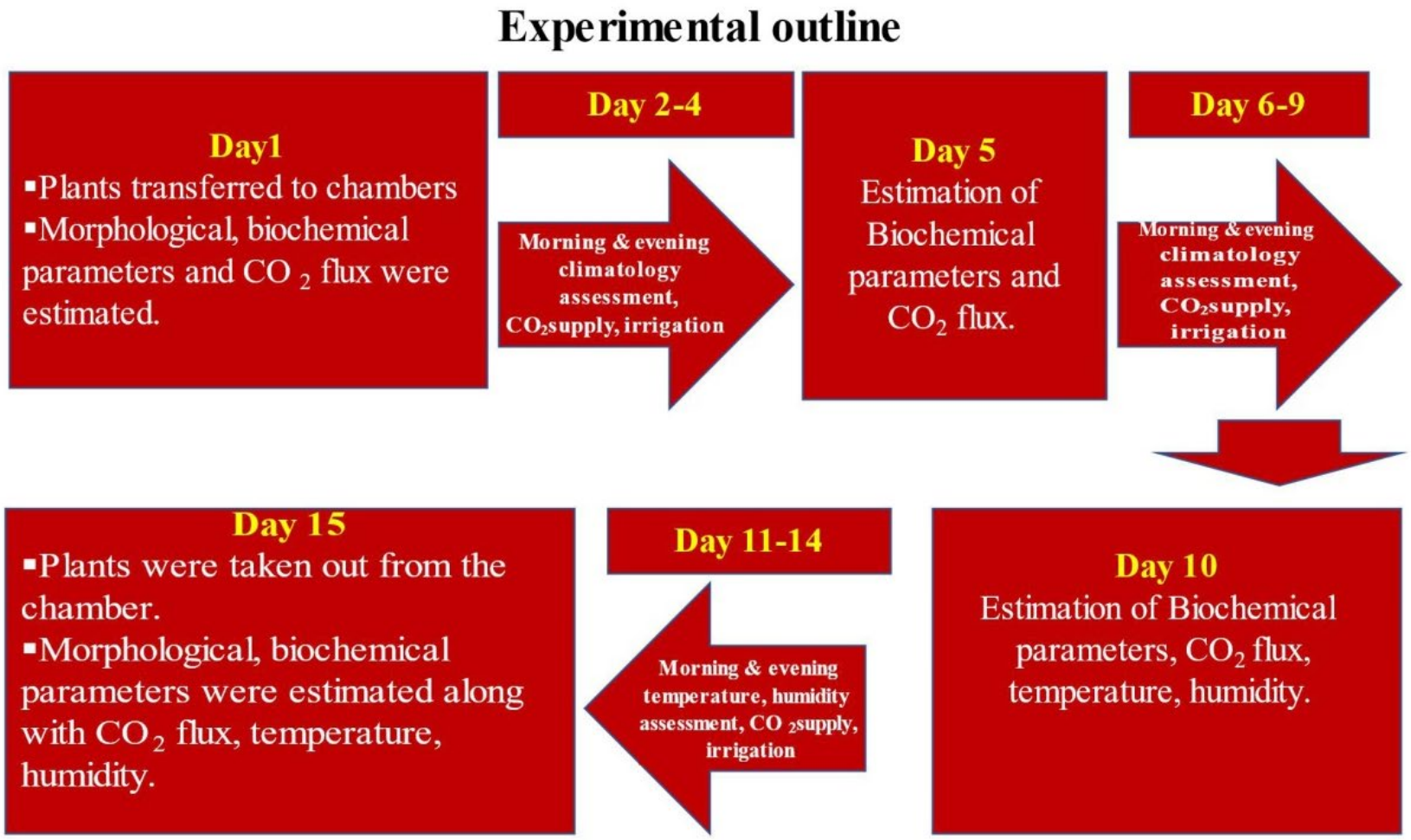


Figure 2.3.1: Experimental outline

Figure 2.3.2: Experimental studies using various tree species (as outlined in Chapter I).

The growth and biochemical responses of individual species subjected to varying concentrations of CO_2 supply have been studied in this chapter (Chapter II).

2.3.1 Growth measurements

Plant height

Plant height of *T. arjuna*, *S. macrophylla*, *P. pinnata*, *S. glauca*, *M. elengi,* and *S. cumini* were assessed at two stages of experimentation, one on the initial day (0DOT) before introducing the plant into the chambers and the other on the final day of treatment (15DOT). Plant height (cm) was assessed from the level of soil to the region of active meristem, using a measuring tape. Above ground, below ground (sacrificing representative plants), and the total height of all six plants were recorded. Mean values were then calculated.

Stem diameter

Stem diameter (cm) was measured from a typical height (collar level) from all the plants using a screw gauge. Reading in screw gauge was taken by observing the main scale reading and the circular scale reading. Reading in a circular scale is calculated from the number of divisions in a circular scale and the least count of screw gauge (0.001cm). The total reading is calculated by adding the main scale and circular scale reading.

Leaf length

Small, medium, and large leaves of plants were selected and marked. The length of the leaf (cm) was measured from end to end. The mean values were calculated from the small, medium, and large leaves.

Leaf breadth

The breadth of the selected leaves of plants (small, medium, large) was measured from tip to tip at the widest portion of the lamina. The breadth of leaves is measured in such a way that the measurement from the widest portion and the middle of the leaf is taken. The mean values are then calculated.

Leaf number

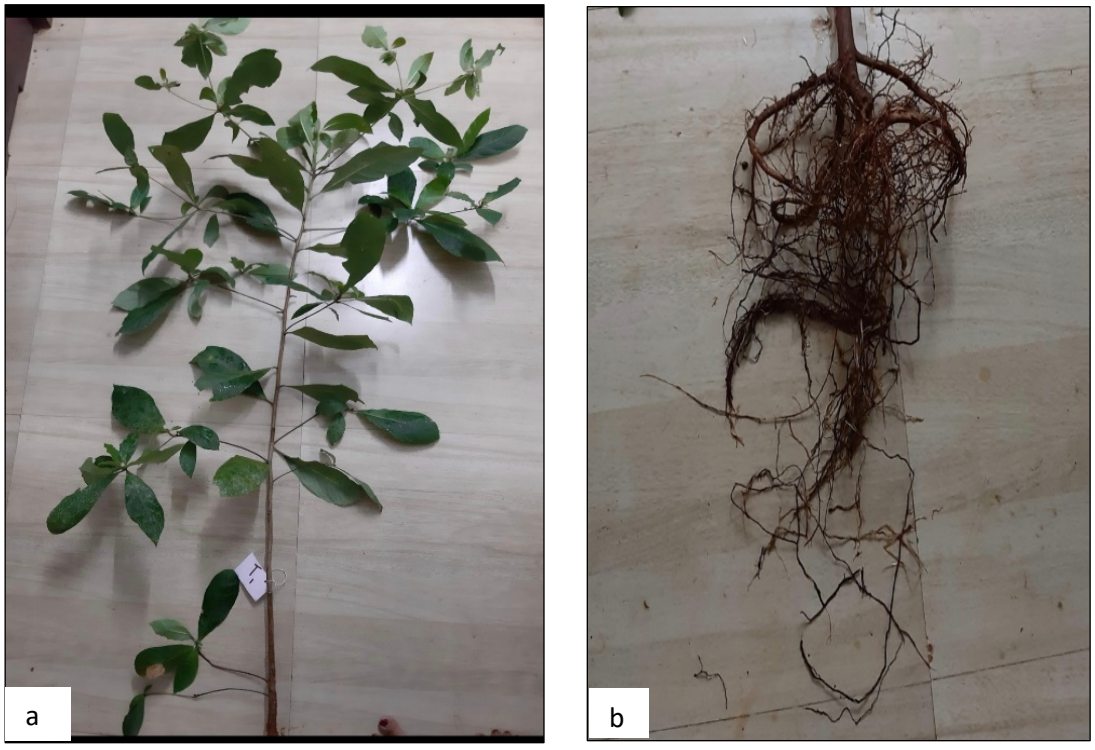
The leaf number was calculated by counting the total number of leaves in the plant. Both the young and old leaves of the plants were counted.

Leaf area

Leaf area (m^2) was calculated using leaf length, breadth, number of leaves, and leaf area constant of dicots. The equation used for leaf area = leaf length leaf breadth×number of leaves× leaf area constant for dicots (0.8). The mean values were calculated and expressed in m^2.

2.3.2 Plant biomass

To estimate the plant biomass for 1DAT, one set from the ambient condition was uprooted. The fresh and dry weight of above-ground and below-ground parts were determined **(Figure 2.3.3).** Total biomass was calculated from the above-ground and below-ground weights of plants. On the final day of experimentation (15DAT), the representative plants from the control and treatment chambers were uprooted, washed, cleaned, and blotted, and the fresh weight of the above-ground and below-ground parts of the plants was determined. The dry weight of the above-ground and below-ground parts of the plants was determined by keeping them in an oven for 24 hrs at 60°C. The total biomass of the control and treated plant was calculated from the above-ground and below-ground parts of the plant.




c


d


e


f


g


h

Figure 2.3.3: Above ground (a,c,e,g,i,k) and below ground (b,d,f,h,j,l) part of *T. arjuna*, *S. macrophylla, P. pinnata, S. glauca, M. elengi,* and *S. cumini*.

2.3.3 Plant moisture content

For the estimation of moisture content, fresh weights of respective plant materials were obtained. The same materials were then subjected to the estimation of dry weight using an oven, which was retained at a constant temperature of 60^0 C. The samples were retained, till they maintained constant weight. Moisture (%) was calculated as the fresh weight of the plant leaf- the dry weight of the plant leaf / fresh weight × 100.

2.3.4 Estimation of Biochemical Parameters

Changes in the biochemical parameters associated with *T. arjuna*, *S. macrophylla*, *P. pinnata, S. glauca, M. elengi,* and *S. cumini,* consequent to CO_2 treatments were recorded at 4 stages (0DOT, 5DOT, 10DOT, and 15DOT). The methodology followed is detailed below:

Estimation of Pigments

The DMSO method has been followed for the analysis of pigments. For this, 0.025 g of the leaf tissue is taken in each test tube and added 7 ml of DMSO reagent. The test tubes were kept in the dark and then in an oven for 1 hour at 60°C. The test tubes were taken from the oven and kept for cooling in darkness. Absorbance was measured using a spectrophotometer at varying ODs. Chlorophyll fragments were calculated using the following equations:

Chlorophyll a = ((12.7×OD 663) - (2.69×OD 645)) ×V×DF/1000×W×1
Chlorophyll b= ((22.9 ×OD 645) – (4.68× OD 663)) × V×DF/ 1000×W×1
Total chlorophyll= ((20.2 × OD 645) + (8.02 × OD 663)) ×V×DF/ 1000×W ×1
Carotenoids= OD 480 + ((0.114×OD 663)- (0.638× OD 645)) × V× DF/ 1000×W×1
Where V is the volume of the solution, W is the weight of the plant, and DF is the dilution factor.

Estimation of Carbohydrates

Carbohydrate was estimated following Dubois *et al*. (1956). For estimation, 0.1g of leaf samples from each treatment were hydrolyzed with 0.5 mL of 2.5N HCl for three hours in a boiling water bath. After cooling to room temperature, the samples were neutralized with sodium carbonate powder until the effervescence stopped. The neutralized samples were diluted to a volume of 100 mL and centrifuged at 4000

rpm for 5 min. 0.5 mL of the supernatant from each sample was taken in separate test tubes and the final volume was made up to 1 mL with distilled water. Then prepared the standards with 0.2, 0.4, 0.6, 0.8, and 1 ml glucose. Made up the volume to 1ml in all the test tubes including the sample by adding distilled water. To the tubes, 4 mL of anthrone reagent was added and heated for 8 min. They were rapidly cooled by keeping the test tube holder in a tray having water. Absorbance at 630 nm was measured using a UV-visible spectrophotometer (Shimadzu, Japan). The concentration of carbohydrate was calculated (mg/g) using the equation:

$$\frac{OD\ of\ sample \times average\ concentration\ of\ standard\ \times total\ volume\ \times DF}{Average\ OD\ of\ standard\ \times Aliquot\ volume\ \times weight\ of\ sample}$$

Protein

Protein was estimated using the method of Lowry *et al*. (1951). For this, 0.5 g of fresh leaf samples were homogenized in 10 ml of phosphate buffer using a mortar and pestle. The homogenate was centrifuged at 6000 rpm for 15 min. The supernatant was used for protein estimation. For the preparation of standards, pipetted out 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard into the test tubes (BSA). Pipetted out 0.1 ml sample extract into the test tube. Make up the volume to 1 ml in all the test tubes by adding distilled water. A tube with 1 ml water served as the blank. To each test tube of standards and blank, 5ml of reagent C (2% Na_2CO_3 and 0.1 N NaOH added with an appropriate concentration of 0.5 % copper sulphate and 1% potassium sodium tartarate) was added. Mixed well and allowed to stand for 10 min. 0.5 ml of reagent D (folin phenol reagent diluted to a 1:1 ratio with distilled water) was added. Mixed well and incubated at room temperature in the dark for 30 minutes. The resultant blue colour was read at 660 nm. The concentration of protein (mg/g) was calculated using the equation:

$$\frac{OD\ of\ sample \times average\ concentration\ of\ standard\ \times total\ volume\ \times DF}{Average\ OD\ of\ standard\ \times Aliquot\ volume\ \times weight\ of\ sample}$$

Phenol

Phenol content was estimated by the procedure of Malick and Singh (1980), for which leaf tissue weighing 0.5 g was homogenized in 5 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water.

Different aliquots were pipetted out and the volume in each tube was made up to 3.0 ml with distilled water. To make the catechol standard, 0.01 g of catechol was mixed with 10 ml of distilled water. Folin-Ciocalteau reagent (0.5ml) was added after 3 minutes, followed by 2 ml of 20% sodium carbonate. The tubes were mixed and placed in a boiling water bath for exactly one minute. They were then cooled, and the absorbance was read at 650nm in a spectrophotometer against a reagent blank. The Concentration of phenol (mg/g) was calculated using the equation:

$$\frac{OD\ of\ sample \times average\ concentration\ of\ standard\ \times total\ volume\ \times DF}{Average\ OD\ of\ standard\ \times Aliquot\ volume\ \times weight\ of\ sample}$$

2.3.5 Estimation of minerals

Samples of leaves were oven-dried at 65°C. Then, a 0.25 g dried sample was taken in a Kjeldahl flask (100 ml) for acid digestion with 60% of 0.25 ml perchloric acid, 1.25 ml nitric acid, and 0.125 ml sulfuric acid. This was then heated at low temperature and digested for 10-15 minutes after the appearance of white fumes. This was then cooled, filtered, and made up to 25 ml after several washings of the filter paper.

Calcium (EDTA titration method)

For calcium estimation, 2.5 ml of acid-digested solution from each sample was taken in a conical flask. 50 ml of distilled water and 5 ml of sodium hydroxide (1.0 N) solution were added to it, followed by 100-200 mg of murexide indicator. This was then titrated using 0.01 M EDTA solution until the pink colour changed to purple. The following equation was used for estimating the percentage of calcium in the solution:

Ca (%) = A× 400.8×V/ v×10000 ×S

A = Volume of EDTA used

V= Total volume of ash solution

v = Volume of ash solution titrated

S = Weight of plant tissue in g

Magnesium (EDTA titration method)

For the estimation of magnesium, 2.5 ml of acid-digested solution was taken and diluted with 50 ml of distilled water. This was then added with 7.5 ml buffer

solution and 100-200 mg of Eriochrome black T indicator. The solution was titrated with EDTA (0.01 M) until the colour changed to blue at the endpoint. The percentage of magnesium was calculated as follows:

Mg (%) = (B-A) × 400.8×V/ v×1.645×10000 ×S

A = Volume of EDTA for determining calcium alone

B = Volume of EDTA for calcium and magnesium

V= Total volume of ash solution

v= Volume of ash solution titrated

S= Weight of plant material taken (mg/g)

Sodium and Potassium

Sodium and potassium content in the leaf samples were estimated using a flame photometer (Systronics, 128). For this, acid-digested solutions of respective leaf samples were taken. Standards of sodium were prepared by weighing 0.252 g sodium in 100 ml distilled water (1000 ppm). This was then proportionately diluted to make working standards. Similarly, standards of potassium were prepared by weighing 1.907g of potassium in 100 ml of distilled water (1000 ppm). This was also diluted adequately to make working standards. The respective standards were used to calibrate the instrument. Samples were then aspirated to get the respective results in ppm levels.

Carbon and Nitrogen

For determining the carbon and nitrogen content of the plants under experimentation, leaves were collected at 0DOT and 15DOT and were then dried and powdered to a uniform size. The carbon and nitrogen contents of each sample were measured using a CHNS organic elemental analyzer, FLASH 2000 (Thermo Scientific).

Plant micronutrients

For determining the copper and zinc content of plants, oven-dried leaf samples were subjected to acid digestion and processed using an AAS (Atomic Absorption Spectrophotometer, Shimadzu, Model AA7000).

2.3.6 Spectral comparison using Fourier- Transform Infrared Spectroscopic Analysis (FTIR)

Infrared analysis of all the plant samples was carried out using the facility available at CSIF, Calicut University. The instrument used is a Cary 620 from Agilent Technologies with high spatial resolution, a large field of view, and chemical imaging. About 1 mg of dried powder from the leaves of *T. arjuna, S. macrophylla, P. pinnata, S. glauca, M. elengi,* and *S. cumini* were mixed with KBr salt and crushed to form a pellet of uniform size and infrared spectra for each of the plant materials were recorded at room temperature in the mid-infrared region of 4000-500 $cm^{-}1$.

2.3.7 Estimation of soil characteristics

Soil moisture

The fresh weight of soil samples from the control and treatment sets was estimated. Their dry weight was estimated by heating them to 60˚C in an oven for a certain amount of time until they attained constant weight. Percentage of soil moisture content was calculated as $\frac{\text{Fresh weight of soil} - \text{dry weight of soil}}{\text{Fresh weight of soil}} \times 100$

Soil pH

Soil solution was prepared (1:10 ratio) and the soil pH was estimated using a digital pH meter (MK V1).

Soil carbon

Organic carbon content associated with soils of respective tree species from the control and CO_2-treated sets was assessed using the Walkley and Black method (Krishnan and Bharathi, 2009). For this, 1.0 g of dried soil sample was taken in a conical flask. This was added with 10 ml of 1N potassium dichromate and 20 ml of concentrated sulfuric acid having silver sulphate dissolved in it, and mixed by swirling. Allowed to stand for 30 minutes and after the reaction was over, it was diluted by adding 200 ml of distilled water. 10 ml of phosphoric acid and 1 ml of diphenylamine indicator were added to it. The colour was changed to bluish-purple, and this was titrated with ferrous ammonium sufate until the blue colour changed to brilliant green. Organic Carbon (%) was calculated as:

percentage of organic carbon = % C × 1.724

V1 = Volume of potassium dichromate

V2 = Volume of ferrous ammonium sulphate

W = Weight of sample.

Available Nitrogen

The available nitrogen in the soil is estimated by the Alkaline Permanganate method. For this, a known weight of soil (20 g) is treated with excess alkaline permanganate and distilled. Organic matter present in the soil is oxidized by nascent oxygen liberated by $KMnO_4$ in the presence of NaOH and thus ammonia is released. This released ammonia is absorbed in a known volume of boric acid (2%) containing double indicator and converted to ammonium borate. This ammonium borate is titrated against standard 0.02 N H_2SO_4.

$$\text{Available nitrogen (Kg/ha)} = \frac{(\text{Volume of } H_2SO_4 \times \text{Normality of } H_2SO_4 \times 0.04 \times 10^6 \times 2.24)}{Weight\ of\ Soil}$$

Available phosphorous

The Bray 1 method was used for the estimation of phosphorous in the soil. Monocalcium phosphate and other acid-soluble forms of P can be extracted using NH_4F and HCl. The unique ability of the fluoride ion to combine Al++ and Fe++ ions in acidic solutions results in the release of P that these trivalent ions were holding in the soil. By using colorimetry, the amount of phosphorus released into the soil solution is calculated as accessible phosphorous.

For the present study, five grams of soil were weighed and then placed in a 100 ml plastic shaking container. Bray 1 extractant (50 ml) was added to it. A blank was also run at the same time. Following a minute of vigorous shaking of the items in a shaker, it was filtered using a Whatman No. 40 filter paper. 5 ml of the filtrate was pipetted into a 25 ml volumetric flask. Diluted the solution in a volumetric flask to 20 ml using distilled water and added 4 ml of reagent B (ammonium molybdate and antimony potassium tartrate, dissolved in H_2SO_4 and added with ascorbic acid) and brought the volume up to 25 ml. After shaking the contents, the colour took time to develop and the resulting intensity of colour was at 660 nm. Available phosphorous in Kg/ha $= Concentration\ of\ phosphorous\ in\ ppm \times \frac{Volume\ of\ reagent}{weight\ of\ soil} \times \frac{make\ up\ volume}{aliquote\ taken}\ X\ 2.24$

Available potassium

The ammonium acetate method is used for the estimation of available potassium in soil. The concentration of potassium ions in the solution was determined using a flame photometer. For this, 5 g of each of the soil samples was transferred into a polythene-shaking bottle. After adding 25 ml of neutral normal ammonium acetate solution, the mixture was shaken for five minutes using a mechanical shaker. The contents were poured into a vial, and filtered using Whatmann No. 40 filter paper. The filtrates were then fed to the flame photometer to obtain the results. Available potassium in soil (Kg/ha) = $Concentration\ of\ potassium\ in\ ppm \times \frac{Volume\ of\ reagent}{weight\ of\ soil} \times 2.24$

2.3.8 Statistical analysis

All statistical tests were done using R statistical software version 4.3.0 (R core team, 2022). Appropriate test of normality and homogeneity was checked using the Shapiro-Wilk normality test and F test. A 2 sample t-test was performed to test significant differences between the control and treatment in the growth and biochemical parameters of plants using R statistical software. Changes in the growth and biochemical parameters between the initial and final days of the treatments were used for the analysis. Kruskal Wallis rank sum test was conducted using stats package version 4.2.2 to test significant differences between the plants under elevated CO_2 conditions. A pairwise comparison was done using Dunn's test. Wilcoxon rank sum exact test was performed to test if there was a significant difference between the change in soil characters between the initial and final day of treatment between control and treatment. The correlation coefficient among morphological, biochemical, and minerals was calculated using Pearson's correlation. The correlogram was constructed using R statistical software, correlation version 0.8.4 (Makowski *et al*., 2020). A biplot for principal component analysis (PCA) was constructed using R statistical software (Le *et al*., 2008).

2.4. RESULTS

2.4.1. Plant height

The height of all the plants increased under elevated CO_2 compared to the control, while a reverse trend was observed in *S. glauca* where the plant height decreased

under CO_2 enrichment compared to control. *T. arjuna* grown in the treated chamber (TC) for 15 days reported significantly higher plant height (158.12 ± 6.86 cm), which was 6.23% higher compared to the initial day of treatment. Plant height of *S. macrophylla* (172.87±7.215cm), *P. pinnata* (192.75±9.91), *M. elengi* (125 ±33.60 cm), *S. cumini* (161±21.63) in TC increased by 8.386 %, 6.05 %, 0.536 %, 3.87 % respectively in the final day of treatment compared to initial day. The highest percentage of increase in plant height in the TC compared to the control chamber (CC) is observed in *T. arjuna* followed by *P. pinnata*, *S. cumini*, *S. macrophylla*, *M. elengi* while in contrast to this, in *S. glauca* 0.543 % decrease in plant height is observed in TC (115.25 ± 4.349 cm) compared to CC (116±15.23 cm) **(Table 2.4.1 and Figure 2.4.1)**. Statistically, the Shapiro-Wilk normality test and F test proved that the data is normally distributed. Further, the results of the 2 sample t-tests showed that $p > 0.05$ **(Annexure 4)**. Thus, there was no significant difference between the treatments. The box plot was represented statistically to show the difference between the treatments **(Figure 2.4.1 a).** The change in plant height between all the plants was done using the Kruskal Wallis rank sum test and reported that there was a significant difference between the treatments of plants. ($H = 13.38$, $df = 5$, $p < 0.05$). **Annexure 5** depicts the Kruskal Wallis chi-square (H) and p-value.

Table 2.4.1: Variation in the Plant height (cm) under elevated levels of CO_2

Control	**D1**	**D15**	**Treatment**	**D1**	**D15**
Terminalia arjuna					
C1	153	165.2	**T1**	136	152
C2	140	141	**T2**	155.5	167
C3	174.2	174.2	**T3**	153.5	160
C4	150	151.5	**T4**	150.5	153.5
Avg	154.300	157.975	**Avg**	148.875	158.125
SD	14.384	14.669	**SD**	8.826	6.860
% Change		+2.382	**% Change**		+6.213
Swietenia macrophylla					
C1	166	180	**T1**	167	172
C2	144	151.5	**T2**	145	183
C3	168	170	**T3**	163	170.5

C4	144	165	**T4**	163	166
Avg	155.500	166.625	**Avg**	159.500	172.875
SD	13.304	11.856	**SD**	9.849	7.215
% Change		+7.154	**% Change**		+8.386
Pongamia pinnata					
C1	181	183	**T1**	178	198
C2	169.5	178	**T2**	197	204
C3	196	196	**T3**	185	186
C4	151	160	**T4**	167	183
Avg	174.375	179.250	**Avg**	181.750	192.750
SD	18.988	14.908	**SD**	12.580	9.912
% Change		+2.796	**% Change**		+6.052
Simarouba glauca					
C1	136.5	138	**T1**	120	121
C2	108	108	**T2**	113	113
C3	104	104	**T3**	111	111
C4	112	114	**T4**	116	116
Avg	115.125	116.000	**Avg**	115.000	115.250
SD	14.619	15.232	**SD**	3.916	4.349
% Change		+0.760	**% Change**		+0.217
Mimusops elengi					
C1	109	110	**T1**	93	93
C2	103	103	**T2**	121	122
C3	140	140	**T3**	159	160
Avg	117.333	117.667	**Avg**	124.333	125.000
SD	19.858	19.655	**SD**	33.126	33.601
% Change		+0.284	**% Change**		+0.536
Syzygium cumini					
C1	145	150	**T1**	150	155
C2	140	143	**T2**	138	143
C3	135	138	**T3**	177	185
Avg	140.000	143.667	**Avg**	155.000	161.000
SD	5.000	6.028	**SD**	19.975	21.633
% Change		+2.619	**% Change**		+3.871

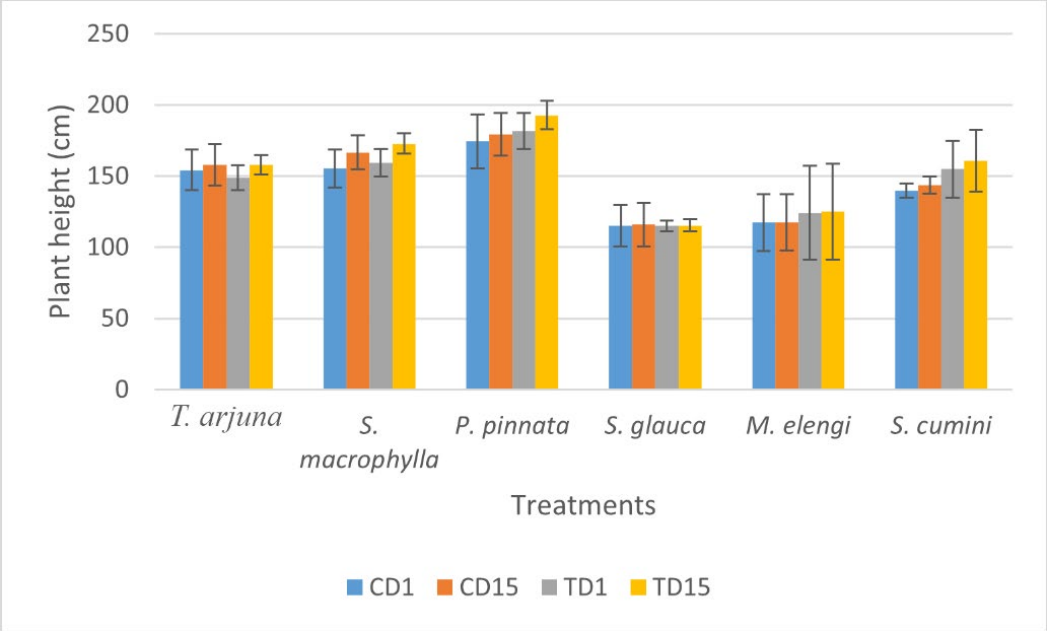


Figure 2.4.1: Effects of elevated CO_2 on the height of plants

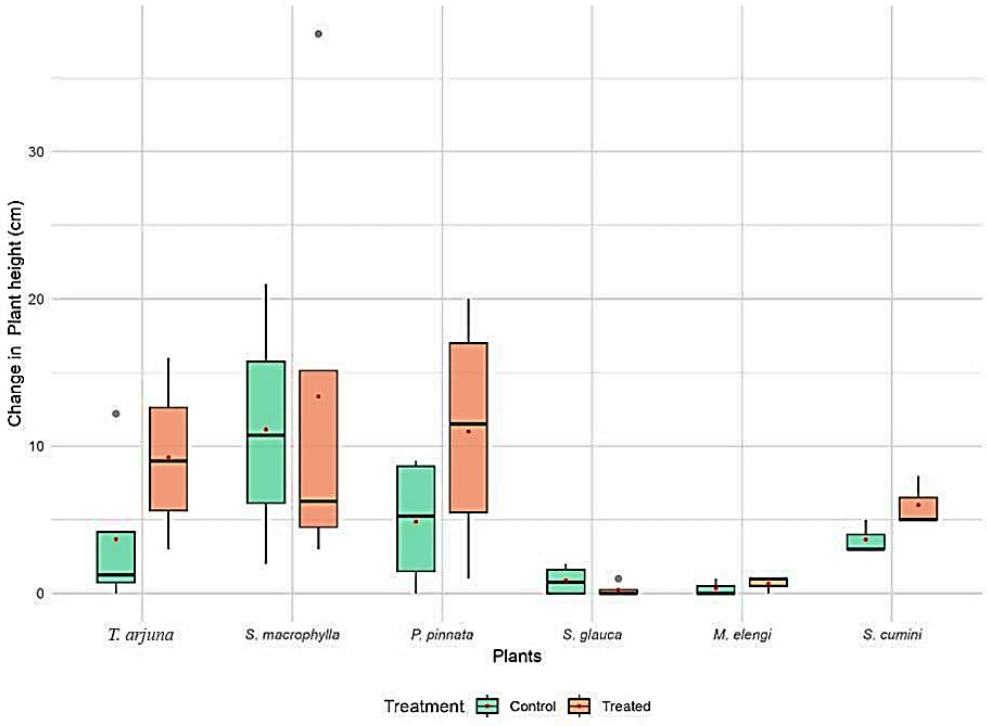


Figure 2.4.1a Box plot representing the change in plant height

2.4.2. Stem diameter

Upon comparing the stem diameter, it is observed that a higher percentage of change is observed in TC compared to CC in all the plants. However, the percentage change

varies according to plants. The higher percentage of change in stem diameter (cm) is observed in TC of *S. glauca* (2.053±0.017) which is 3.819% higher compared to control (0.024±1.370) followed by *M. elengi* where the difference between the percentage change in control (0.095±1.385) and treatment (0.194±2.773) is 1.388% followed by *S. cumini* which is 1.285% followed by *T. arjuna* which is 1.049%. The mean Stem diameter of *S. cumini* from CC and TC after 15 days is 1.490 ± 0.244 and 1.560 ± 0.061 respectively. The mean Stem diameter of *T. arjuna* is observed to be 1.54 ±0.138 and1.515±0.165 from CC and TC respectively. In *S. macrophylla* mean stem diameter is found to be 1.813±0.197 in CC and 1.925±0.167 in TC respectively. The minimum difference in the percentage change between control and treatment is observed to be in *S. macrophylla* which is 0.067% **(Table 2.4.2 and Figure 2.4.2).** Shapiro Wilk normality test and F test were carried out and found that data is non-normally distributed in *S. glauca*, *M. elengi,* and *T. arjuna* since $p < 0.05$. 2 sample t-test **(Annexure 4)** proved there was no significant difference between the treatments ($P > 0.05$) in all the plants which is represented in the box plot (**Figure 2.4.2 a**). The results of the Kruskal Wallis rank sum test report that a significant difference in stem diameter between the treatments of plants is absent **(Annexure 5)**.

Table 2.4.2: Variation in Stem diameter of plants (cm) under elevated levels of CO_2

Control	**D1**	**D15**	**Treatment**	**D1**	**D15**
Terminalia arjuna					
C1	1.51	1.51	**T1**	1.22	1.34
C2	1.34	1.37	**T2**	1.39	1.41
C3	1.53	1.58	**T3**	1.67	1.67
C4	1.7	1.7	**T4**	1.64	1.64
Avg	1.52	1.54	**Avg**	1.48	1.515
SD	0.147	0.138	**SD**	0.214	0.165
% Change		+1.316	**% Change**		+2.365
Swietenia macrophylla					
C1	2.18	2.09	**T1**	1.63	1.83
C2	1.29	1.74	**T2**	1.71	1.74
C3	1.78	1.63	**T3**	2.09	2.09
C4	1.79	1.79	**T4**	2.04	2.04
Avg	1.76	1.813	**Avg**	1.868	1.925
SD	0.365	0.197	**SD**	0.231	0.167
% Change		+2.983	**% Change**		+3.050
Pongamia pinnata					
C1	1.59	1.67	**T1**	1.48	1.48
C2	1.53	1.56	**T2**	1.58	1.58

C3	1.66	1.66	**T3**	1.63	1.63
C4	1.58	1.58	**T4**	1.66	1.66
Avg	1.59	1.617	**Avg**	1.588	1.588
SD	0.054	0.056	**SD**	0.079	0.079
% Change		+1.250	**% Change**		0
Simarouba glauca					
C1	2.05	2.02	**T1**	2.05	2.05
C2	2.08	2.07	**T2**	2.045	2.07
C3	1.85	2.03	**T3**	1.84	2.06
C4	2.05	2.02	**T4**	1.87	2.03
Avg	2.008	2.035	**Avg**	1.951	2.053
SD	0.106	0.024	**SD**	0.112	0.017
% Change		+1.370	**% Change**		+5.189
Mimusops elengi					
C1	1.27	1.31	**T1**	1.25	1.39
C2	1.12	1.12	**T2**	1.03	1.01
C3	1.22	1.23	**T3**	1.29	1.27
Avg	1.203	1.220	**Avg**	1.190	1.223
SD	0.076	0.095	**SD**	0.140	0.194
% Change		+1.385	**% Change**		+2.773
Syzygium cumini					
C1	1.38	1.38	**T1**	1.51	1.53
C2	1.77	1.77	**T2**	1.49	1.52
C3	1.29	1.32	**T3**	1.59	1.63
Avg	1.480	1.490	**Avg**	1.530	1.560
SD	0.255	0.244	**SD**	0.053	0.061
% Change		+0.676	**% Change**		+1.961

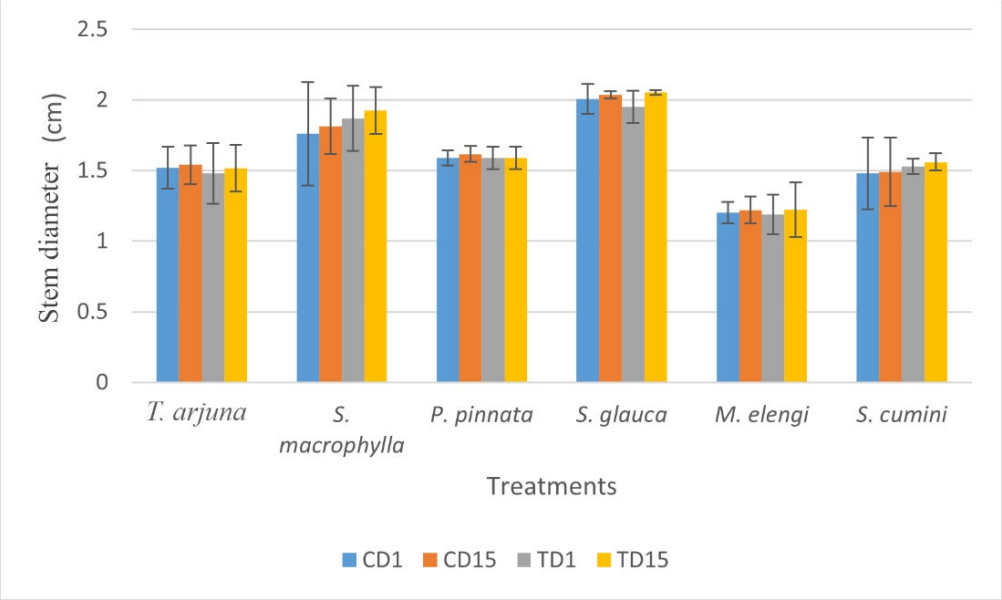


Figure 2.4.2: Effects of elevated CO_2 on stem diameter of plants

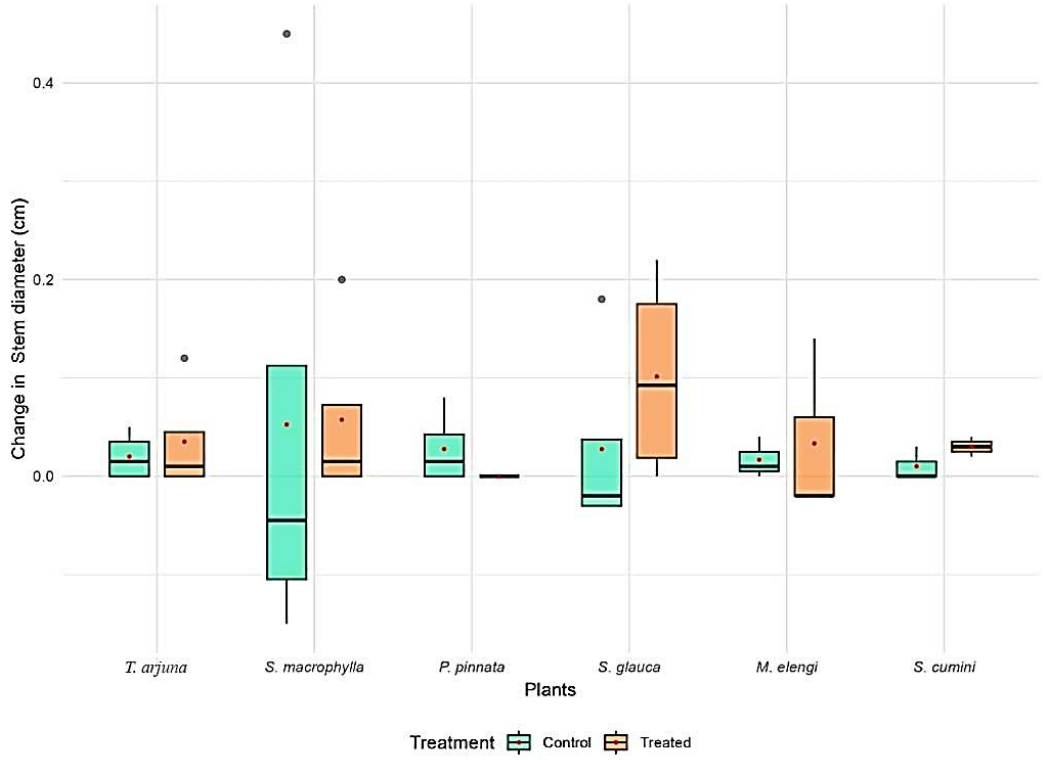


Figure 2.4.2a Box plot representing the change in stem diameter

2.4.3. Leaf length

Leaf length of *S. glauca*, *S. macrophylla*, and *S. cumini* increased under elevated CO_2 compared to the control. *S. glauca* grown in TC recorded significantly higher leaf length (7.98±0.957 cm) compared to that grown at CC (7.438±0.534 cm). The leaf length of *S. macrophylla* inside TC is 15.838±1.147 cm and that of CC is 16.275±2.074 cm. In *S. cumini* leaf length inside TC is reported to be 12.92 ±1.086 cm which is higher compared to that of CC (12.507±2.21). In contradiction to this, the increase in leaf length of *T. arjuna*, *P. pinnata,* and *M. elengi* on the 15th day of CO_2 treatment is lower compared to that of control. In control chambers of *T. arjuna, P. pinnata,* and *M. elengi* leaf length increased by 4.47%, 5.62%, and 4.68% on the 15th day compared to the initial day. In the Treated chambers of *T. arjuna*, *P. pinnata,* and *M. elengi* leaf length increased by 2.92%, 2.80%, and 3.14% on the 15th day compared to the initial. As far as plants are considered, *S. glauca* shows a higher percentage of increase in leaf length followed by *S. cumini*, *S. macrophylla*, *M. elengi, T. arjuna,* and *P. pinnata* (**Table 2.4.3).** Two sample t-tests were carried out to test the difference in leaf length in control and treatment. In *S. macrophylla* a significant difference was observed [t (6) = -3.137, $p < 0.05$] between control (16.27±2.07) and treatment (15.83±1.14). Similarly in *S. glauca,* a significant

difference was observed [t (6) = -4.072, p < 0.05] between control (7.438± 0.53) and treatment (7.98± 0.957) (**Figure 2.4.3 , Annexure 4**). Results of the Kruskal Wallis rank sum test indicate that a significant difference in leaf length between the treatments of plants was present (H =5.187, df = 5, p < 0.5) **(Annexure 5)**.

Table 2.4.3: Variation in the Leaf length (cm) of plants under elevated levels of CO_2

Control	**D1**	**D15**	**Treatment**	**D1**	**D15**
Terminalia arjuna					
C1	17.1	17.9	**T1**	15.96	16.96
C2	15.83	16.3	**T2**	15.8	15.8
C3	17.6	18.13	**T3**	16.9	17.53
C4	15.6	16.76	**T4**	14.3	14.53
Avg	16.53	17.273	**Avg**	15.74	16.205
SD	0.97	0.883	**SD**	1.076	1.3286
% Change		+4.470	**% Change**		+2.920
Swietenia macrophylla					
C1	14.16	14.16	**T1**	14.26	14.27
C2	16.6	16.61	**T2**	16.23	16.27
C3	19	19	**T3**	16.93	16.98
C4	15.33	15.33	**T4**	15.76	15.83
Avg	16.273	16.275	**Avg**	15.795	15.838
SD	2.073	2.074	**SD**	1.1306	1.147
% Change		+0.0012	**% Change**		+0.272
Pongamia pinnata					
C1	12.73	12.76	**T1**	9.4	9.8
C2	11.83	13.4	**T2**	12.23	12.66
C3	12.16	13.35	**T3**	10.73	10.83
C4	13.1	13.1	**T4**	11.9	12.2
Avg	12.455	13.153	**Avg**	11.065	11.373
SD	0.568	0.293	**SD**	1.283	1.305
% Change		+5.620	**% Change**		+2.80
Simarouba glauca					
C1	6.66	6.9	**T1**	7.3	7.63
C2	7.4	7.46	**T2**	7.73	8.16
C3	7.23	7.23	**T3**	6.66	6.93
C4	8.16	8.16	**T4**	8.86	9.2
Avg	7.363	7.438	**Avg**	7.638	7.98
SD	0.619	0.534	**SD**	0.926	0.957
% Change		+0.950	**% Change**		+4.580
Mimusops elengi					
C1	11.63	11.63	**T1**	11.83	12.9
C2	11.1	12.73	**T2**	12.46	12.46
C3	12.46	12.5	**T3**	11.93	12
Avg **SD**	11.73 0.685	12.287 0.58	**Avg** **SD**	12.073 0.339	12.453 0.45
% Change		+4.680	**% Change**		+3.140

Syzygium cumini					
C1	9.96	9.96	**T1**	13.5	14.13
C2	13.5	13.56	**T2**	12.03	12.03
C3	13.73	14	**T3**	12.6	12.6
Avg	12.397	12.507	**Avg**	12.71	12.92
SD	2.113	2.216	**SD**	0.741	1.086
% Change		+0.880	**% Change**		+1.650

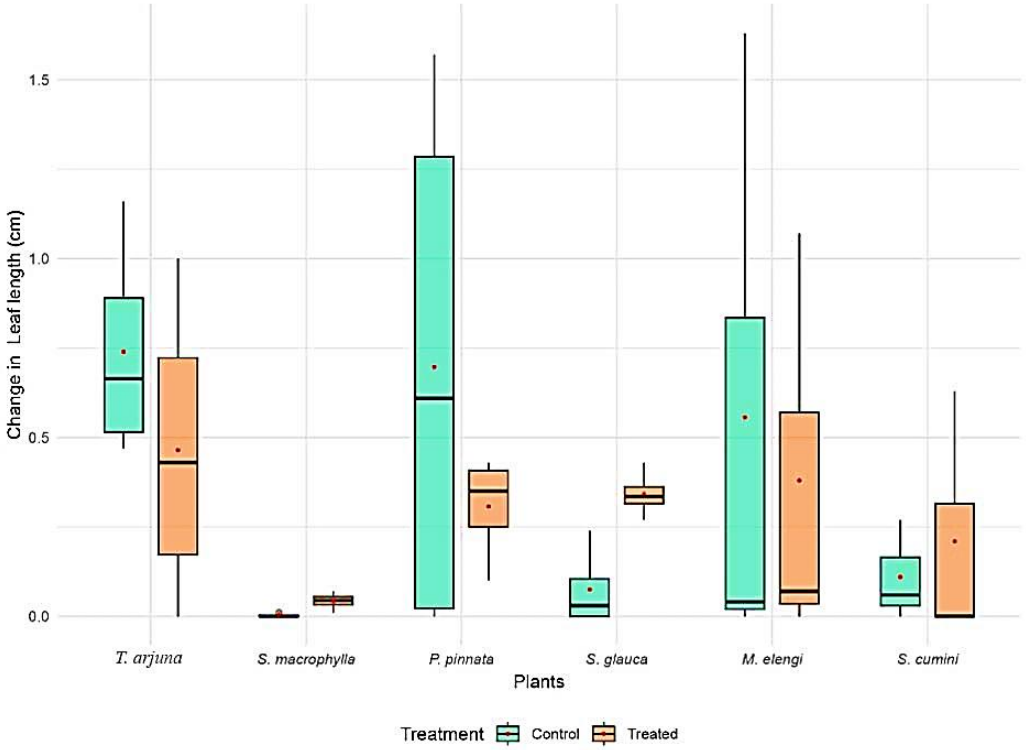


Figure 2.4.3: Box plot representing the change in leaf length

2.4.4. Leaf breadth

Leaf breadth of *S. glauca*, *T. arjuna, S. macrophylla,* and *M. elengi* increased under elevated CO_2 compared to the control. Leaf breadth (cm) of *S. glauca* from TC on the final day of the experiment is 2.38 ±0.223 which is 8.67% higher compared to that of the initial day (2.19 ±0.156) while in CC on the final day of the experiment, leaf breadth is 2.273±0.294 which is 1.337% higher compared to that of the initial day (2.243 ± 0.299). The leaf breadth of *T. arjuna* inside TC on the final day of the experiment is 5.723±0.377 which is 7.474% higher compared to that of the initial day (5.325±0.395) while in CC leaf breadth on the final day of the experiment is 5.673±0.654 which is 6.735% higher compared to that of the initial day (5.315±0.67). The leaf breadth of *S. macrophylla* inside TC on the final day of the

experiment is 5.788±0.677 which is 0.573% higher compared to that of the initial day (5.755±0.687) while in CC leaf breadth on the final day of the experiment is 5.373± 0.515 which is 0.336 % higher compared to that of the initial day (5.355±0.544). The leaf breadth of *M. elengi* inside TC on the final day of the experiment is 5.04±0.589 which is 1.143% higher compared to that of the initial day (4.983±0.652) while in CC leaf breadth on the final day of the experiment is 4.41±0.115 which is 1.077% higher compared to that of the initial day (4.363±0.188). In *P. pinnata* and *S. cumini* percentage of increase in leaf breadth on the final day compared to the initial day is more in CC compared to TC. In *P. pinnata* an increase of 2.782% was recorded in CC when exposed to ambient air while the increase in the percentage of leaf breadth in TC is 0.172% which is lower compared to the control. Similarly in *S. cumini* an increase of 4.086% was recorded in control on the final day compared to the initial day while *S. cumini* when exposed to enriched CO_2, reported an increase of 2.304 % in TC when compared to the first day of the experiment. (**Table 2.4.4**). In *S. glauca*, 2 sample t-test proved that there is a significant difference in leaf breadth [t (6) = -2.979, $p < 0.05$] between control (2.273±0.294) and treatment (2.38±0.223) which is represented in the box plot **(Figure 2.4.4, Annexure 4)**. Significant differences in leaf breadth between the treatments of plants were reported (H =15.76, df = 5, $p<0.01$) (**Annexure 5**).

Table 2.4.4. Variation in the Leaf breadth (cm) of plants under elevated levels of CO_2

Control	**D1**	**D15**	**Treatment**	**D1**	**D15**
Terminalia arjuna					
C1	5.6	6.06	**T1**	5.9	6.13
C2	5.1	5.5	**T2**	5	5.26
C3	4.5	4.83	**T3**	5.2	5.9
C4	6.06	6.3	**T4**	5.2	5.6
Avg	5.315	5.673	**Avg**	5.325	5.723
SD	0.67	0.654	**SD**	0.395	0.377
% Change		+6.735	**% Change**		+7.474
Swietenia macrophylla					
C1	4.66	4.73	**T1**	5	5
C2	5.93	5.93	**T2**	5.36	5.46
C3	5.6	5.6	**T3**	6.23	6.23
C4	5.23	5.23	**T4**	6.43	6.46
Avg	5.355	5.373	**Avg**	5.755	5.788
SD	0.544	0.515	**SD**	0.685	0.677
% Change		+0.336	**% Change**		+0.573

Pongamia pinnata					
C1	6	6.16	**T1**	5.03	5.03
C2	6.7	6.7	**T2**	6.16	6.16
C3	6.33	6.7	**T3**	5.76	5.8
C4	6.13	6.3	**T4**	6.2	6.2
Avg	6.29	6.465	**Avg**	5.788	5.798
SD	0.305	0.277	**SD**	0.543	0.542
% Change		+2.782	**% Change**		+0.172
Simarouba glauca					
C1	1.95	2	**T1**	2.4	2.66
C2	2.16	2.2	**T2**	2.13	2.43
C3	2.2	2.2	**T3**	2.03	2.13
C4	2.66	2.69	**T4**	2.2	2.3
Avg	2.243	2.273	**Avg**	2.19	2.38
SD	0.299	0.294	**SD**	0.156	0.223
% Change		+1.337	**% Change**		+8.675
Mimusops elengi					
C1	4.4	4.4	**T1**	4.23	4.36
C2	4.16	4.3	**T2**	5.36	5.36
C3	4.53	4.53	**T3**	5.36	5.4
Avg	4.363	4.41	**Avg**	4.983	5.04
SD	0.188	0.115	**SD**	0.652	0.589
% Change		+1.077	**% Change**		+1.143
Syzygium cumini					
C1	4.06	4.43	**T1**	4.56	4.66
C2	4.83	4.96	**T2**	4.73	4.86
C3	4.03	4.06	**T3**	5.03	5.13
Avg	4.307	4.483	**Avg**	4.773	4.883
SD	0.453	0.452	**SD**	0.238	0.236
% Change		+4.086	**% Change**		+2.304

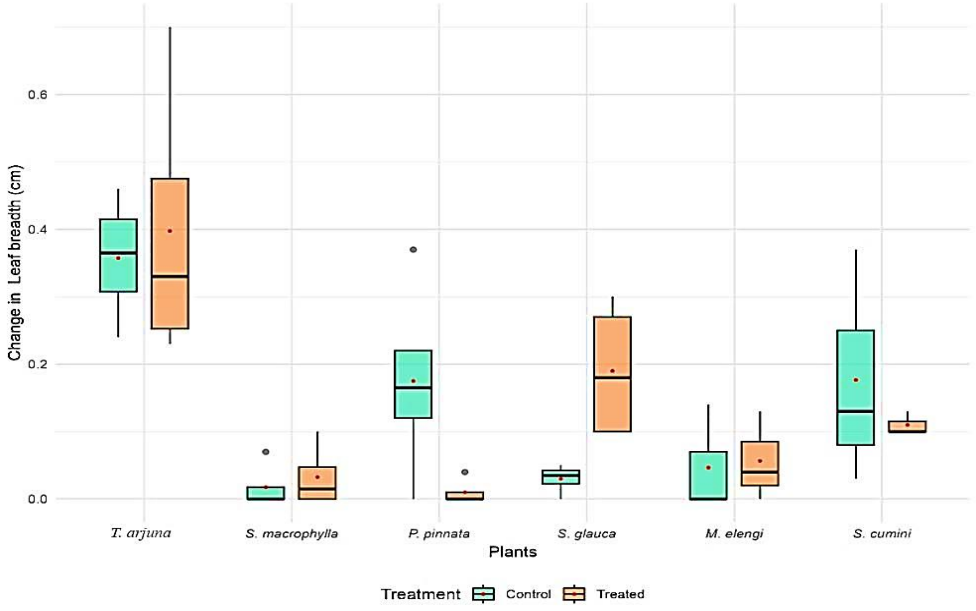


Figure 2.4.4: Box plot representing the change in leaf breadth

2.4.5. Leaf number

The leaf number of *T. arjuna* inside TC on the 15th day of the experiment is 155.75 ±7.45 which is 48.68 % higher compared to that of the 1st day (104.75±20.05). The leaf number of *S. macrophylla* inside TC on the 15th day of the experiment is 192.5 ±20.92 which is 6.796% higher compared to that of the 1st day (180.25 ±22.67). The leaf number of *P. pinnata* inside TC on the 15th day of the experiment is 223.25 ±35.69 which is 49.58 % higher compared to that of the 1st day (149.25 ±81.29). The leaf number of *S. glauca* inside TC on the 15th day of the experiment is 223.25±35.69 which is 49.58 % higher compared to that of the 1st day (149.25±81.29). In all these plants a higher percentage increase in leaf number is observed in treatment compared to control. A higher percentage increase in TC compared to CC was observed in *T. arjuna* followed by *P. pinnata*, *S. glauca* followed by *S. macrophylla*. 2 sample t-test showed that there was a significant difference in leaf number [t (6) = -3.732, $p < 0.05$] between the control (85.25±28.28) and treatment (155.75± 7.45) in *T. arjuna* (**Annexure 4**). Shapiro-Wilk normality test and F test proved that the data is normally distributed since $p > 0.05$ in all the plants **(Figure 2.4.5).** In *S. cumini* leaf number inside TC on the 15th day of the experiment is 138.33±17.55 which is 10.08% higher than on the 1st day (125.66±18.33). In CC leaf number on the 15th day is 141± 26.51 which is 10.44% higher compared to that of the 1st day (127.66±22.50). The results are depicted in **Table 2.4.5**. Kruskal Wallis rank sum test was carried out between the plants and reported that there was a significant difference between the treatments of plants [H =11.43, df = 5, $p < 0.05$] (**Annexure 5**).

Table 2.4.5: Variation in the Leaf number of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Terminalia arjuna					
C1	102	105	**T1**	96	165
C2	54	50	**T2**	108	157
C3	86	111	**T3**	131	154
C4	82	75	**T4**	84	147
Avg	81	85.25	**Avg**	104.75	155.75
SD	19.967	28.289	**SD**	20.056	7.455
% Change		+5.246	**% Change**		+48.687
Swietenia macrophylla					
C1	134	148	**T1**	149	163
C2	179	185	**T2**	187	200

C3	182	192	**T3**	182	195
C4	171	182	**T4**	203	212
Avg	166.5	176.75	**Avg**	180.25	192.5
SD	22.159	19.619	**SD**	22.677	20.92
% Change		+6.156	**% Change**		+6.796
Pongamia pinnata					
C1	144	180	**T1**	115	173
C2	69	151	**T2**	241	255
C3	120	140	**T3**	55	225
C4	90	100	**T4**	186	240
Avg	105.75	142.75	**Avg**	149.25	223.25
SD	32.989	33.12	**SD**	81.291	35.669
% Change		+34.98	**% Change**		+49.58
Simarouba glauca					
C1	144	180	**T1**	115	173
C2	69	151	**T2**	241	255
C3	120	140	**T3**	55	225
C4	90	100	**T4**	186	240
Avg	105.75	142.75	**Avg**	149.25	223.25
SD	32.989	33.12	**SD**	81.291	35.669
% Change		+35.98	**% Change**		+49.58
Mimusops elengi					
C1	183	133	**T1**	118	90
C2	86	63	**T2**	160	150
C3	131	143	**T3**	127	110
Avg	133.333	113	**Avg**	135	116.667
SD	48.542	43.589	**SD**	22.113	30.551
% Change		-15.28	**% Change**		-13.58
Syzygium cumini					
C1	110	115	**T1**	140	155
C2	120	140	**T2**	105	120
C3	153	168	**T3**	132	140
Avg	127.667	141	**Avg**	125.667	138.333
SD	22.502	26.514	**SD**	18.339	17.559
% Change		+10.44	**% Change**		+10.08

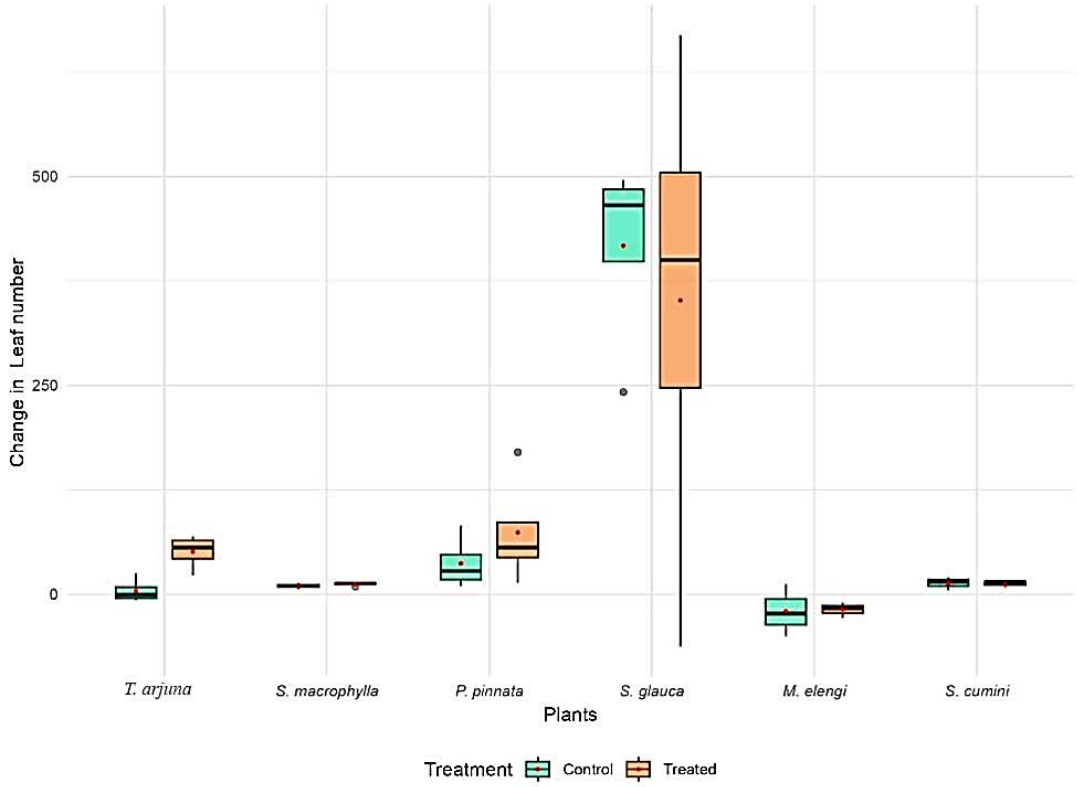


Figure 2.4.5: Box plot representing the change in leaf number

2.4.6. Leaf area

T. arjuna and *P. pinnata* when exposed to elevated CO_2 conditions, recorded significantly higher leaf area in TC compared to CC. The mean leaf area of *T. arjuna* inside TC on the 15th day is 1.162 ±0.194 which is 64.35 % higher compared to that of the first day (0.707±0.173) whereas in CC on the 15th day, the leaf area recorded is 0.670±0.237 which is 16.72% higher compared to that of the first day (0.574±0.18). The mean leaf area of *P. pinnata* from TC on the 15th day is 1.214±0.403 which is 49.14 % higher compared to that of the first day (0.814±0.555) whereas in CC on the 15th day, the leaf area recorded is 0.97±0.213 which is 47.19% higher compared to that of the first day (0.659±0.192). The mean leaf area of *S. macrophylla* inside TC on the 15th day is 1.434±0.361 which is 7.819 % higher compared to that of the first day (1.33±0.35) whereas in CC there is a reduction of leaf area by 6.04% compared to initial day. In *S. glauca* from TC, the mean leaf area was noticed to be 1.329±0.35 which is 82.82% higher compared to that of the initial day (0.722±0.232) while in CC mean leaf area on the 15th day is observed to be 1.195±0.345 which is 94.62% higher compared to initial day

(0.614±0.228). In *M. elengi* leaf area decreased by 11.57% in CC and 10.16% in TC on the 15th day of the experiment compared to the initial day. A reduction of 1.41% in leaf area is noticed in TC compared to CC. The mean leaf area of *S. cumini* inside TC on the 15th day is 0.7 ± 0.129 which is 14.37% higher compared to that of the first day (0.612 ± 0.116) whereas in CC on the 15th day, the leaf area recorded is 0.641± 0.204 which is 15.91% higher compared to that of the first day (0.553±0.173), (**Table 2.4.6 and Figure 2.4.6**). Two sample t-tests were carried out to test the difference in leaf area between control and treatment. In *T. arjuna* significant difference was observed [t (6) = -4.155, $p < 0.05$] between control (0.67±0.237) and treatment (1.162±0.194) (**Figure 2.4.6 a, Annexure 4).** Significant differences in leaf area between the treatments of plants were reported (H = 14.095, df = 5, $p < 0.05$) (**Annexure 5**).

Table 2.4.6: Variation in the Leaf area (m^2) of plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Terminalia arjuna					
C1	0.7814	0.911	**T1**	0.723	1.372
C2	0.3488	0.359	**T2**	0.683	1.044
C3	0.5449	0.778	**T3**	0.921	1.274
C4	0.6202	0.634	**T4**	0.5	0.957
Avg	0.574	0.670	**Avg**	0.707	1.162
SD	0.180	0.237	**SD**	0.173	0.194
% Change		+16.72	**% Change**		+64.35
Swietenia macrophylla					
C1	0.707	0.793	**T1**	0.85	0.93
C2	1.410	1.458	**T2**	1.301	1.421
C3	1.549	1.634	**T3**	1.536	1.65
C4	1.097	1.167	**T4**	1.646	1.734
Avg	1.191	1.263	**Avg**	1.33	1.434
SD	0.374	0.368	**SD**	0.35	0.361
% Change		-6.040	**% Change**		+7.819
Pongamia pinnata					
C1	0.880	1.132	**T1**	0.435	0.682
C2	0.438	1.085	**T2**	1.452	1.591
C3	0.739	1.002	**T3**	0.272	1.131
C4	0.578	0.660	**T4**	1.098	1.452
Avg	0.659	0.970	**Avg**	0.814	1.214
SD	0.192	0.213	**SD**	0.555	0.403603
% Change		+47.19	**% Change**		+49.14
Simarouba glauca					
C1	0.374	0.945	**T1**	0.659	1.331
C2	0.499	1.144	**T2**	0.632	1.823

C3	0.687	0.995	**T3**	0.535	1.116
C4	0.896	1.696	**T4**	1.06	1.046
Avg	0.614	1.195	**Avg**	0.722	1.329
SD	0.228	0.345	**SD**	0.232	0.35095
% Change		+94.62	**% Change**		+82.82
Mimusops elengi					
C1	0.749	0.544	**T1**	0.472	0.405
C2	0.318	0.276	**T2**	0.855	0.801
C3	0.592	0.648	**T3**	0.65	0.57
Avg	0.553	0.489	**Avg**	0.659	0.592
SD	0.218	0.192	**SD**	0.191659	0.198915
% Change		-11.57	**% Change**		-10.16
Syzygium cumini					
C1	0.356	0.406	**T1**	0.689	0.816
C2	0.626	0.753	**T2**	0.478	0.561
C3	0.677	0.764	**T3**	0.669	0.724
Avg	0.553	0.641	**Avg**	0.612	0.700
SD	0.173	0.204	**SD**	0.116	0.129
% Change		+15.91	**% Change**		+14.37

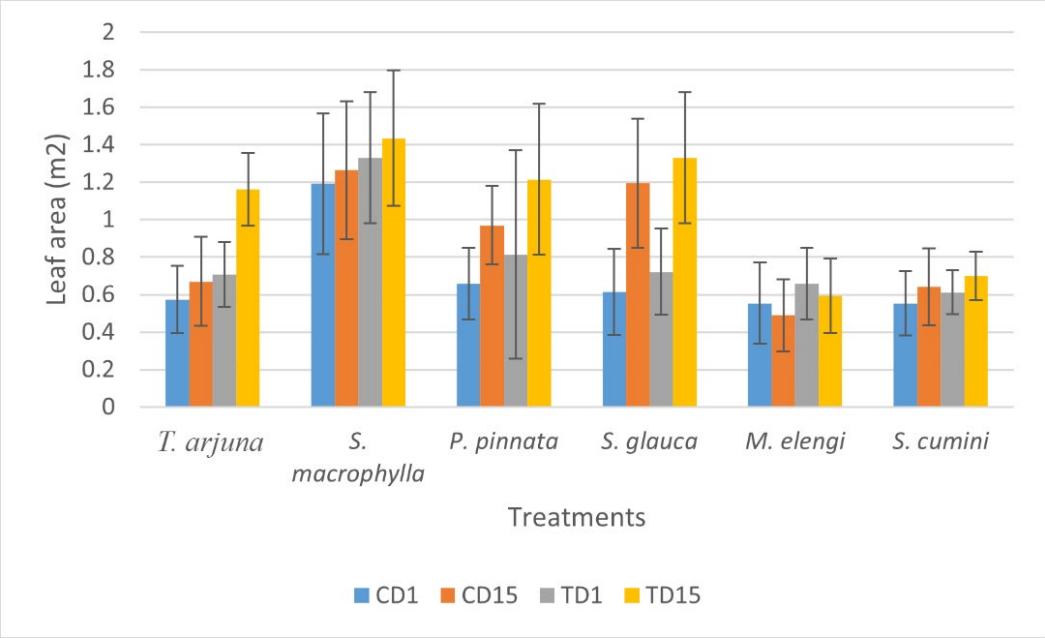


Figure 2.4.6: Effects of elevated CO_2 on leaf area of plants

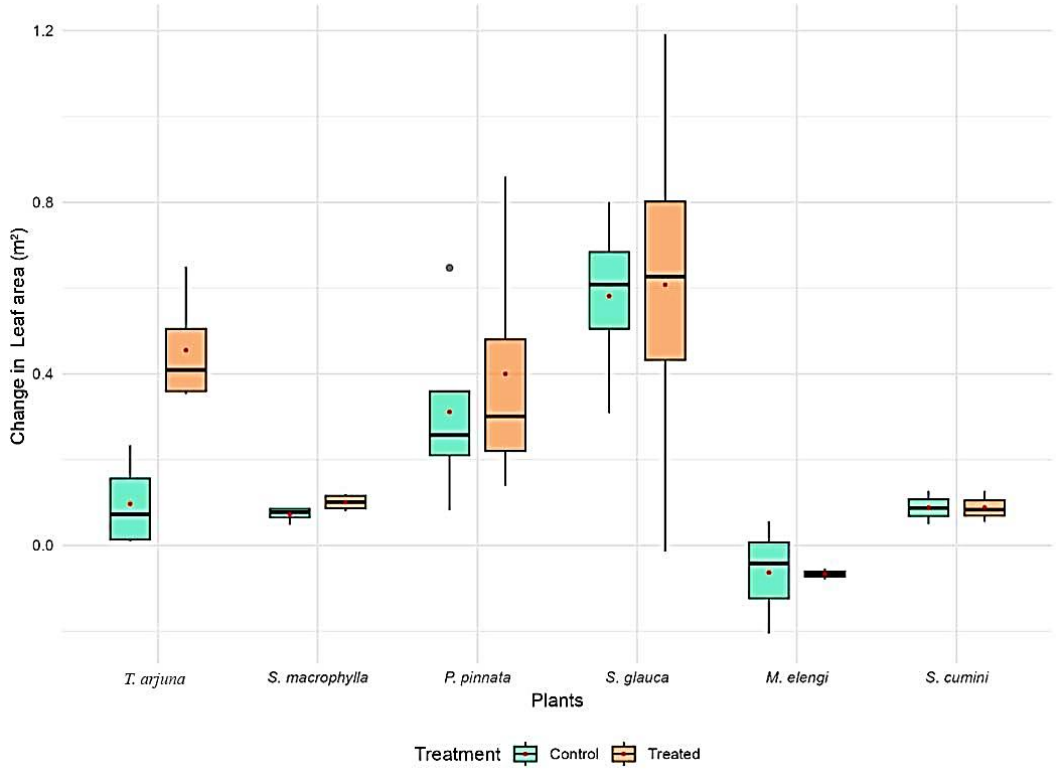


Figure 2.4.6a: Box plot representing the change in leaf area

2.4.7. Plant biomass

The biomass of plants increased under enriched CO_2 conditions compared to the control except for *M. elengi*. The increase in biomass was highest in *S. glauca* followed by *S. macrophylla*, *S. cumini*, *T. arjuna,* and *P. pinnata* **(Figure 2.4.7)**. *S. macrophylla* consumed a higher share of CO_2 compared to the other plants under elevated CO_2 and hence consumption of CO_2 by 1.0 kg of the plant is calculated and found to be 628.57 ppm considering the fact that 700 g of the plant consumes 440 ppm CO_2. D.F. indicates the day flux of CO_2. The Biomass Enhancement Ratio (BER), which is the ratio between the total plant biomass of CO_2-treated plants and those grown at control levels, is how we measure the CO_2 response and is calculated and found to be highest in *S. glauca* followed by *S. macrophylla*, *T. arjuna*, *S. cumini*, *P. pinnata* and *M. elengi* which is given below in **table 2.4.7**.

Table 2.4.7: Biomass Enhancement Ratio of Plants.

Plant	D.F (TC) (ppm)	Total Biomass (g)		BER
		CC	TC	
T. arjuna	396	175	200	1.14
S. macrophylla	440	125	175	1.4
P. pinnata	390	200	225	1.125
S. glauca	348.13	275	400	1.45
M. elengi	181.73	125	125	1
S. cumini	188.83	100	125	1.25

(BER- Biomass Enhancement Ratio), (D.F- Day flux)

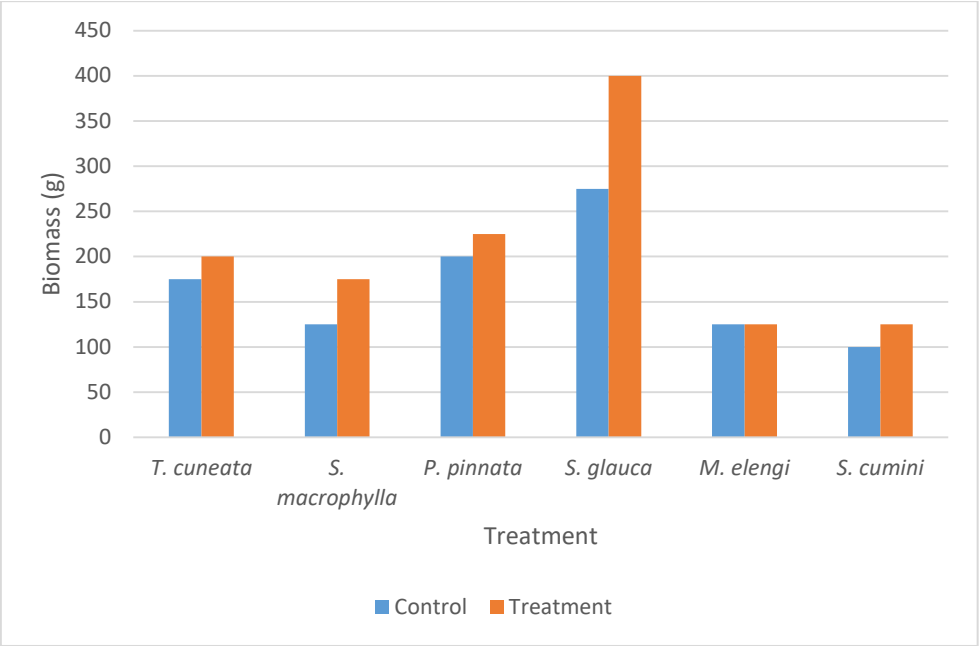


Figure 2.4.7: Effects of elevated CO_2 on the biomass of plants

2.4.8 Plant moisture content

Plant moisture content increased in *P. pinnata* and *S. glauca* by 4.155% and 7.8% under enriched CO_2 conditions compared to control after 15 days of CO_2 treatment which might be due to the reduced transpiration and water use. In *M. elengi* an increase in plant moisture content by 1.36 % on the 15th day of CO_2 treatment compared to the first day is observed while in EC a reduction of 0.26% in plant moisture is observed compared to CC in *M. elengi*. In *T. arjuna*, *S. macrophylla,* and *S. cumini*, the percentage change in plant moisture decreased by 5.42%, 1.97%, and 6.92% under enriched CO_2 conditions compared to the control **(Table 2.4.8).**

Table 2.4.8: Variations in Plant moisture (%) under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Terminalia arjuna					
C1	71.761	71.578	**T1**	72.027	71.095
C2	68.551	68.836	**T2**	71.466	66.796
C3	73.053	69.089	**T3**	70.742	63.960
C4	72.008	68.473	**T4**	70.326	67.300
Avg	71.343	69.494	**Avg**	71.140	67.288
SD	1.944	1.412	**SD**	0.756	2.933
% Change		-2.590	**% Change**		-5.420
Swietenia macrophylla					
C1	65.891	63.964	**T1**	60.000	61.3208
C2	65.517	61.062	**T2**	57.759	64.4628
C3	64.800	63.063	**T3**	64.615	58.0357
C4	65.714	61.682	**T4**	65.068	58.7719
Avg	65.481	62.443	**Avg**	61.861	60.6478
SD	0.479	1.315	**SD**	3.567	2.9068
% Change		-4.640	**% Change**		-1.9700
Pongamia pinnata					
C1	64.2	66.45	**T1**	59.23	62.33
C2	60.23	64.25	**T2**	58.45	60.23
C3	65.66	67.23	**T3**	56.78	59.36
C4	64.34	66.34	**T4**	58.98	61.22
Avg	63.608	66.068	**Avg**	58.360	60.785
SD	2.346	1.275	**SD**	1.102	1.280
% Change		+3.867	**% Change**		+4.155
Simarouba glauca					
C1	55.233	61.538	**T1**	51.825	52.294
C2	58.273	55.556	**T2**	53.216	56.436
C3	56.216	51.020	**T3**	56.725	56.436
C4	54.110	52.586	**T4**	59.694	73.585
Avg	55.958	55.175	**Avg**	55.365	59.687
SD	1.767	4.640	**SD**	3.547	9.468
% Change		-1.390	**% Change**		+7.800
Mimusops elengi					
C1	63.415	63.333	**T1**	61.202	61.905
C2	61.765	63.087	**T2**	61.314	61.875
C3	61.538	63.314	**T3**	63.684	64.972
Avg	62.239	63.245	**Avg**	62.067	62.917
SD	1.024	0.137	**SD**	1.402	1.779
% Change		+1.620	**% Change**		+1.360
Syzygium cumini					
C	69.173	64.539	**T1**	65.493	59.864
C2	1.508	63.636	**T2**	65.672	62.346
C3	62.602	59.064	**T3**	67.213	62.416
Avg	67.761	62.413	**Avg**	66.126	61.542
SD	4.618	2.935	**SD**	0.946	1.454
% Change		-36.440	**% Change**		-6.920

2.4.9. Plant pigments and metabolites

2.4.9.1 Plant Pigments

S. macrophylla grown in the treated chamber (TC) for 15 days reported significantly higher chlorophyll-a content (2.55±0.801) which was 42.28 % higher compared to that of the first day (1.795±0.532). *S. macrophylla* grown in the control chamber (CC) for 15 days reported significantly higher chlorophyll-a content (3.103±0.34) which was 37.3 % higher compared to that of the first day (2.26±0.325). *P. pinnata* grown in the TC for 15 days reported significantly higher chlorophyll-a content (4.158±0.875) which was 20.17 % higher compared to that of the first day (3.46±1.387). *P. pinnata* grown in the CC for 15 days reported significantly higher chlorophyll-a content (3.473±0.852) which was 15.38 % higher compared to that of the first day (3.01±0.877). In *T. arjuna*, there is a reduction of chlorophyll-a content by 20.41% in TC compared to a reduction of 20.57% in CC. In *S. glauca* an increase of 22.25% is reported in CC while only an increase of 9.419% is noticed in TC. An increase of 157.43% in chlorophyll a is reported in CC while a 13.52% reduction in TC is noticed in *M. elengi*. In *S. cumini*, an increase of 109.29 % in chlorophyll-a is reported in CC compared to an increase of 34.5% in TC **(Table 2.4.9).** 2 sample t-tests proved a significant difference in chlorophyll-a [t (4) = 4.913, p < 0.05] between the control (1.99± 0.044) and treatment (1.49±0.276) in *M. elengi* **(Annexure 6)**. Results of the Shapiro-Wilk normality test and F test prove that the data is normally distributed **(Figure 2.4.8).** Significant differences in chlorophyll-a between the plants were reported under elevated CO_2 conditions (H =13.246, df= 5, p < 0.05) (**Annexure 7**).

Table 2.4.9: Variations in Chlorophyll a (mg g^{-1}) of plants under elevated levels of CO_2

Days		**D1**	**D5**	**D10**	**D15**
Terminalia arjuna					
Control	**C1**	3.06	5.28	1.68	2.38
	C2	2.2	3.31	1.08	1.2
	C3	1.64	1.63	2.24	1.91
	C4	2.14	2.24	1.39	1.69
	Avg	2.26	3.115	1.598	1.795
	SD	0.589	1.602	0.493	0.49
	% Change				-20.57
Treatment	**T1**	4.278	3.724	2.277	1.862
	T2	1.92	2.44	2.26	1.42

	T3	2.1	1.71	1.65	1.93
	T4	2.61	1.73	1.05	1.92
	Avg	2.21	1.96	1.653	1.757
	SD	0.358	0.416	0.605	0.292
	% Change				-20.41
Swietenia macrophylla					
Control	**C1**	2.14	3.25	4.01	3.55
	C2	1.93	3.84	4.17	2.81
	C3	2.27	3.16	3.03	2.87
	C4	2.7	3.33	3.1	3.18
	Avg	2.26	3.395	3.578	3.103
	SD	0.325	0.305	0.596	0.34
	% Change				+37.30
Treatment	**T1**	2.3	3.08	4.27	2.47
	T2	2.18	2.21	2.94	2.77
	T3	1.51	3.71	3.68	1.52
	T4	1.19	3.51	3.04	3.45
	Avg	1.795	3.128	3.483	2.553
	SD	0.532	0.666	0.619	0.801
	% Change				+42.28
Pongamia pinnata					
Control	**C1**	3.71	3.27	4.66	3.58
	C2	3.02	2.45	3.71	4.62
	C3	1.77	2.15	1.87	3.02
	C4	3.54	0.44	3.37	2.67
	Avg	3.01	2.078	3.403	3.473
	SD	0.877	1.19	1.158	0.852
	% Change				+15.38
Treatment	**T1**	4.45	4.46	4.22	4.41
	T2	4.73	5.34	4.27	5.05
	T3	1.77	2.48	2.35	2.96
	T4	2.89	3.28	2.92	4.21
	Avg	3.46	3.89	3.44	4.158
	SD	1.387	1.263	0.958	0.875
	% Change				+20.17
Simarouba glauca					
Control	**C1**	0.83	1.23	0.7	0.61
	C2	0.66	0.94	0.63	0.63
	C3	0.98	1.48	1.23	1.21
	C4	0.55	0.55	0.74	1.24
	Avg	0.755	1.05	0.825	0.923
	SD	0.189	0.4	0.274	0.35
	% Change				+22.25
Treatment	**T1**	0.74	1.01	0.84	0.91
	T2	0.66	0.8	0.74	0.79

	T3	0.65	1.59	1.13	0.81
	T4	1.05	1.36	0.7	0.88
	Avg	0.775	1.19	0.853	0.848
	SD	0.188	0.353	0.194	0.057
	% Change				+9.419
Mimusops elengi					
Control	**C1**	0.92	2.7	2.3	2.04
	C2	0.62	1.98	1.19	1.96
	C3	0.78	2.38	1.87	1.97
	Avg	0.773	2.353	1.787	1.99
	SD	0.15	0.361	0.56	0.044
	% Change				+157.43
Treatment	**T1**	2.13	2.17	1.32	1.46
	T2	1.47	2.11	1.81	1.78
	T3	1.57	1.19	1.36	1.23
	Avg	1.723	1.823	1.497	1.49
	SD	0.356	0.549	0.272	0.276
	% Change				-13.52
Syzygium cumini					
Control	**C1**	0.69	1.47	1.31	1.63
	C2	0.5	0.92	1.28	1.01
	C3	0.52	1.44	1.21	0.94
	Avg	0.57	1.277	1.267	1.193
	SD	0.104	0.309	0.051	0.38
	% Change				+109.29
Treatment	**T1**	0.9	0.75	0.89	1.26
	T2	1.05	0.98	1.24	1.28
	T3	0.77	1.28	1.08	1.12
	Avg	0.907	1.003	1.07	1.22
	SD	0.14	0.266	0.175	0.087
	% Change				+34.50

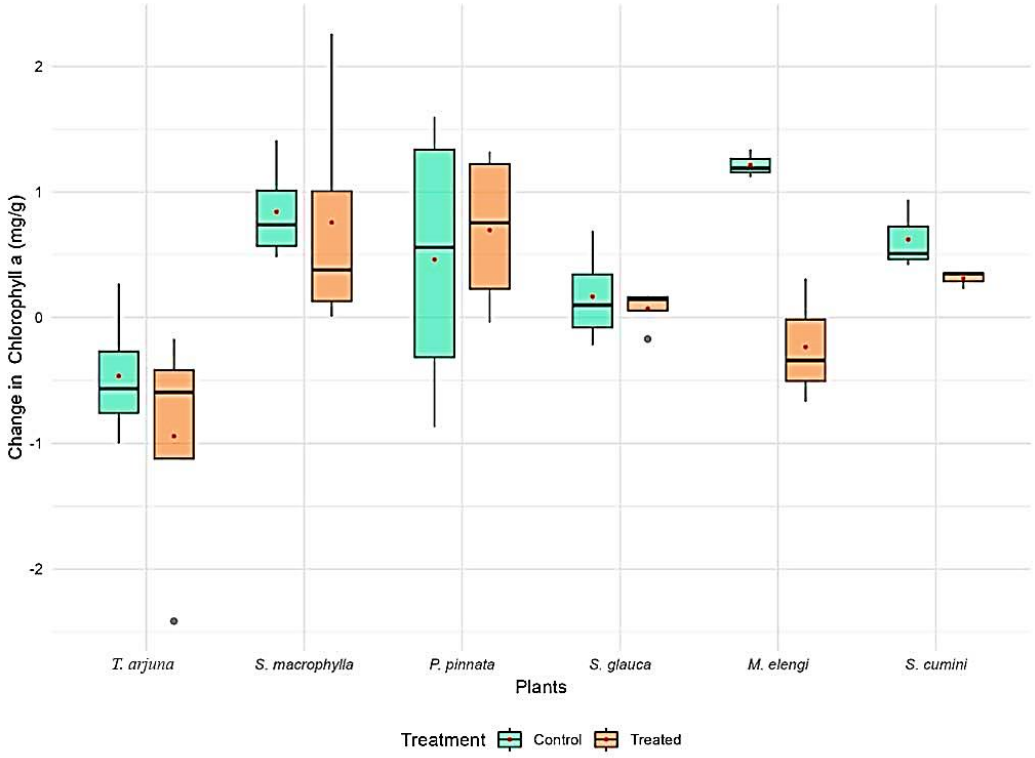


Figure 2.4.8: Box plot representing the change in chlorophyll a

Considering chlorophyll-b, there was an increase in the pigment in TC compared to CC in the case of *S. macrophylla* and *P. pinnata*. In *T. arjuna* a reduction of 46.39% was observed in the pigment in CC and a reduction of 16.86% in TC. In *S. glauca* an increase of 38.75% was observed in the pigment in CC and an increase of 7.638% in TC. In *M. elengi,* an increase of 171.2% in chlorophyll-b is reported in CC while a reduction of 8.259% was noticed in TC. In *S. cumini* in CC, a reduction of 1.12% was noticed while in TC an increase of 67.48% was observed (**Table 2.4.10**). A significant difference in chlorophyll-b between the treatments is not evident in all the plants which are reported from the 2-sample t-test where $p > 0.05$. Shapiro-Wilk normality test and F test prove that the data is normal in distribution, which is illustrated in the box plot (**Figure 2.4.9**). Significant differences in chlorophyll-b between the plants are reported under elevated CO_2 conditions ($H=14.56$, $df=5$, $p < 0.05$) (**Annexure 7**).

Table 2.4.10. Variations in Chlorophyll-b content (mg g^{-1}) of plants under elevated levels of CO_2

Days		D1	D5	D10	D15
Terminalia arjuna					
Control	**C1**	1.19	2.01	0.71	0.72
	C2	0.88	1.2	0.46	0.37
	C3	0.78	0.87	0.75	0.53
	C4	1.03	0.97	0.53	0.46
	Avg	0.97	1.263	0.613	0.52
	SD	0.179	0.517	0.14	0.149
	% Change				-46.39
Treatment	**T1**	1.779	1.431	0.891	0.657
	T2	0.92	1.04	0.85	0.47
	T3	0.89	0.95	0.65	0.62
	T4	0.2	0.7	0.52	0.58
	Avg	0.67	0.897	0.673	0.557
	SD	0.407	0.176	0.166	0.078
	% Change				-16.86
Swietenia macrophylla					
Control	**C1**	0.55	1.27	1.74	1.09
	C2	0.59	1.51	2.11	0.91
	C3	0.64	1.33	1.23	1.19
	C4	0.69	1.28	1.17	0.86
	Avg	0.618	1.348	1.563	1.013
	SD	0.061	0.111	0.446	0.154
	% Change				+63.91
Treatment	**T1**	0.6	1.31	1.8	1.16
	T2	0.57	0.68	1.04	0.98
	T3	0.4	1.24	1.44	0.67
	T4	0.32	1.49	1.26	1.12
	Avg	0.473	1.18	1.385	0.983
	SD	0.135	0.35	0.321	0.222
	% Change				+107.8
Pongamia pinnata					
Control	**C1**	5.87	4.23	10.86	7.35
	C2	4.23	4.32	1.93	7.89
	C3	10.86	1.93	7.89	8.19
	C4	5.46	0.03	6.97	3.45
	Avg	6.605	2.628	6.913	6.72
	SD	2.921	2.055	3.713	2.208
	% Change				+1.741
Treatment	**T1**	6.64	6.3	8.8	8.22
	T2	7.08	9.57	9.29	8.86
	T3	1.68	3.68	5.01	5.22
	T4	3.08	4.9	5.87	7.59
	Avg	4.62	6.113	7.243	7.473
	SD	2.655	2.541	2.12	1.589
	% Change				+61.75

Simarouba glauca					
Control	**C1**	0.29	0.65	0.28	0.3
	C2	0.18	0.44	0.29	0.25
	C3	0.35	1.02	0.42	0.39
	C4	0.21	1.03	0.23	0.49
	Avg	0.258	0.785	0.305	0.358
	SD	0.077	0.29	0.081	0.106
	% Change				+38.75
Treatment	**T1**	0.2	0.34	0.37	0.33
	T2	0.25	0.43	0.36	0.25
	T3	0.25	0.59	0.4	0.33
	T4	0.45	0.58	0.24	0.33
	Avg	0.288	0.485	0.343	0.31
	SD	0.111	0.121	0.07	0.04
	% Change				+7.638
Mimusops elengi					
Control	**C1**	2.04	6.15	4.95	4.43
	C2	1.24	4.47	2.55	4.32
	C3	1.52	5.39	3.9	4.27
	Avg	1.6	5.337	3.8	4.34
	SD	0.406	0.841	1.203	0.082
	% Change				+171.2
Treatment	**T1**	4.58	5.22	2.82	3.24
	T2	3.26	4.65	3.79	3.96
	T3	3.31	2.7	3.03	3.03
	Avg	3.717	4.19	3.213	3.41
	SD	0.748	1.321	0.51	0.488
	% Change				-8.259
Syzygium cumini					
Control	**C1**	0.41	0.12	0.45	0.51
	C2	0.27	0.22	0.44	0.3
	C3	0.39	0.02	0.66	0.25
	Avg	0.357	0.12	0.517	0.353
	SD	0.076	0.1	0.124	0.138
	% Change				-1.120
Treatment	**T1**	0.21	0.31	0.3	0.42
	T2	0.18	0.22	0.38	0.41
	T3	0.34	0.34	0.33	0.39
	Avg	0.243	0.29	0.337	0.407
	SD	0.085	0.062	0.04	0.015
	% Change				+67.48

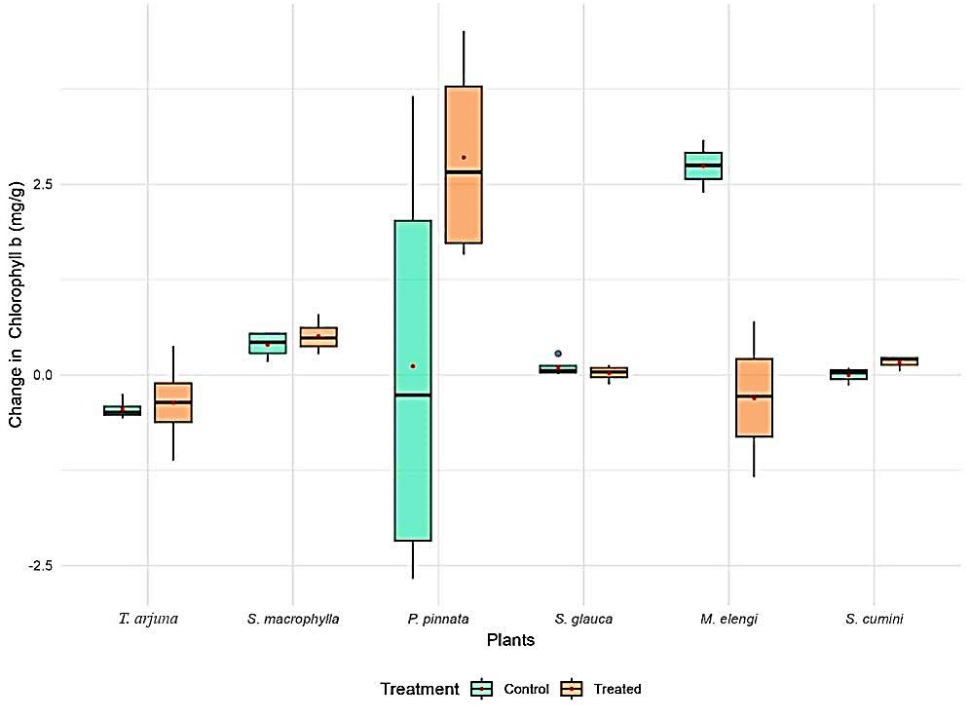


Figure 2.4.9: Box plot representing the change in chlorophyll b

As far as total chlorophyll is concerned, *S. macrophylla* and *P. pinnata* showed an increase of pigment in TC compared to CC. *S. macrophylla* grown in TC for 15 days reported significantly higher total chlorophyll content (3.535±0.989) which was 56.2 % higher compared to that of the first day (2.263±0.661). Total chlorophyll content in the control chamber on the 15th day is (4.113±0.379) which is 42.91% higher compared to that of the initial (2.878±0.379). An increase of 13.29 % is noticed in TC compared to CC. Compared to CC an increase of 13.29% in total chlorophyll content is noticed in TC. *P. pinnata* grown in TC for 15 days reported significantly higher total chlorophyll content (10.85±2.293) which was 43.33 % higher compared to that of the first day (7.573±3.764). Pigment content in the control chamber on the 15th day (8.975±2.687) is 32.47% higher compared to that of the initial (6.775±2.763). Total chlorophyll content in *T. arjuna* decreased by 28.43% and 17.79% in CC and TC, respectively, while in *S. glauca*, a 26.73% increase in total chlorophyll in CC and 9.125% in TC is noticed. In *S. cumini* an increase of 65.91% of total chlorophyll content in CC and an increase of 40.76% in TC is reported. In *M. elengi* 164.09% increase in total chlorophyll content and a decrease of 11.20% are observed in CC and TC respectively (**Table 2.4.11 and Figure 2.4.10**). In *M. elengi*, a significant difference [t (4) = 4.914, $p < 0.05$] in total chlorophyll between

control and treatment is observed (**Annexure 6**). Normal distribution of data is revealed from the results of the F test, where $p > 0.05$ which is illustrated in the box plot (**Figure 2.4.10 a**). Significant differences in total chlorophyll between the plants were reported under elevated CO_2 conditions ($H = 17.26$, $df=5$, $p<0.005$) (**Annexure 7**).

Table 2.4.11. Variations in Total Chlorophyll (mg g^{-1}) of plants under elevated CO_2

Days		**D1**	**D5**	**D10**	**D15**
Terminalia arjuna					
Control	**C1**	4.25	7.28	2.39	3.1
	C2	3.08	4.51	1.54	1.56
	C3	2.42	2.5	2.98	2.43
	C4	3.16	3.2	1.91	2.15
	Avg	3.228	4.373	2.205	2.31
	SD	0.758	2.11	0.623	0.639
	% Change				-28.43
Treatment	**T1**	6.056	5.154	3.167	2.519
	T2	2.84	3.48	3.11	1.89
	T3	2.99	2.66	2.3	2.55
	T4	2.6	2.43	1.57	2.49
	Avg	2.81	2.857	2.327	2.31
	SD	0.197	0.552	0.77	0.365
	% Change				-17.79
Swietenia macrophylla					
Control	**C1**	2.7	4.52	5.75	4.63
	C2	2.51	5.34	6.28	3.72
	C3	2.91	4.49	4.26	4.06
	C4	3.39	4.61	4.26	4.04
	Avg	2.878	4.74	5.138	4.113
	SD	0.379	0.403	1.036	0.379
	% Change				+42.91
Treatment	**T1**	2.89	4.39	6.06	3.63
	T2	2.74	2.89	3.98	3.75
	T3	1.91	4.95	5.12	2.19
	T4	1.51	4.99	4.3	4.57
	Avg	2.263	4.305	4.865	3.535
	SD	0.661	0.982	0.93	0.989
	% Change				+56.20
Pongamia pinnata					
Control	**C1**	8.96	7.03	14.44	10.18
	C2	6.87	4.13	10.8	11.95
	C3	2.85	5.12	5.4	8.03
	C4	8.42	0.4	9.63	5.74
	Avg	6.775	4.17	10.068	8.975
	SD	2.763	2.787	3.725	2.687
	% Change				+32.47
Treatment	**T1**	10.38	10.07	12.14	11.78
	T2	11.05	13.92	12.62	12.99

	T3	3.25	5.76	6.86	7.64
	T4	5.61	7.66	8.19	11.01
	Avg	7.573	9.353	9.953	10.855
	SD	3.764	3.519	2.862	2.293
	% Change				+43.33
Simarouba glauca					
Control	**C1**	1.12	1.88	0.97	0.92
	C2	0.84	1.38	0.92	0.87
	C3	1.32	2.5	1.65	1.6
	C4	0.76	2.73	0.97	1.73
	Avg	1.01	2.123	1.128	1.28
	SD	0.258	0.611	0.349	0.448
	% Change				+26.73
Treatment	**T1**	0.94	1.35	1.21	1.24
	T2	0.91	1.23	1.1	1.05
	T3	0.9	2.18	1.54	1.14
	T4	1.5	1.95	0.95	1.21
	Avg	1.063	1.678	1.2	1.16
	SD	0.292	0.46	0.25	0.084
	% Change				+9.125
Mimusops elengi					
Control	**C1**	1.22	3.64	3.02	2.69
	C2	0.79	2.66	1.56	2.61
	C3	0.98	3.2	2.43	2.6
	Avg	0.997	3.167	2.337	2.633
	SD	0.215	0.491	0.734	0.049
	% Change				+164.09
Treatment	**T1**	2.8	3	1.73	1.94
	T2	1.96	2.8	2.35	2.38
	T3	2.04	1.61	1.81	1.72
	Avg	2.267	2.47	1.963	2.013
	SD	0.464	0.751	0.337	0.336
	% Change				-11.20
Syzygium cumini					
Control	**C1**	1.11	1.59	1.76	2.13
	C2	0.77	1.14	1.72	1.3
	C3	0.91	1.46	1.88	1.2
	Avg	0.93	1.397	1.787	1.543
	SD	0.171	0.232	0.083	0.511
	% Change				+65.91
Treatment	**T1**	1.12	1.05	1.19	1.68
	T2	1.23	1.2	1.61	1.69
	T3	1.11	1.63	1.41	1.5
	Avg	1.153	1.293	1.403	1.623
	SD	0.067	0.301	0.21	0.107
	% Change				+40.76

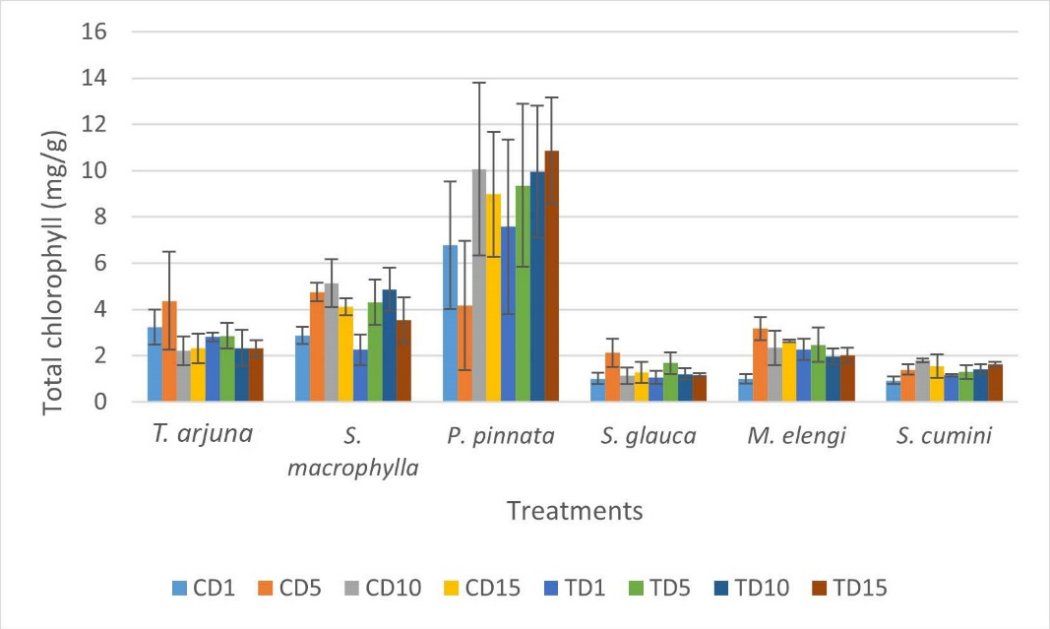


Figure 2.4.10: Effects of elevated CO_2 on total chlorophyll

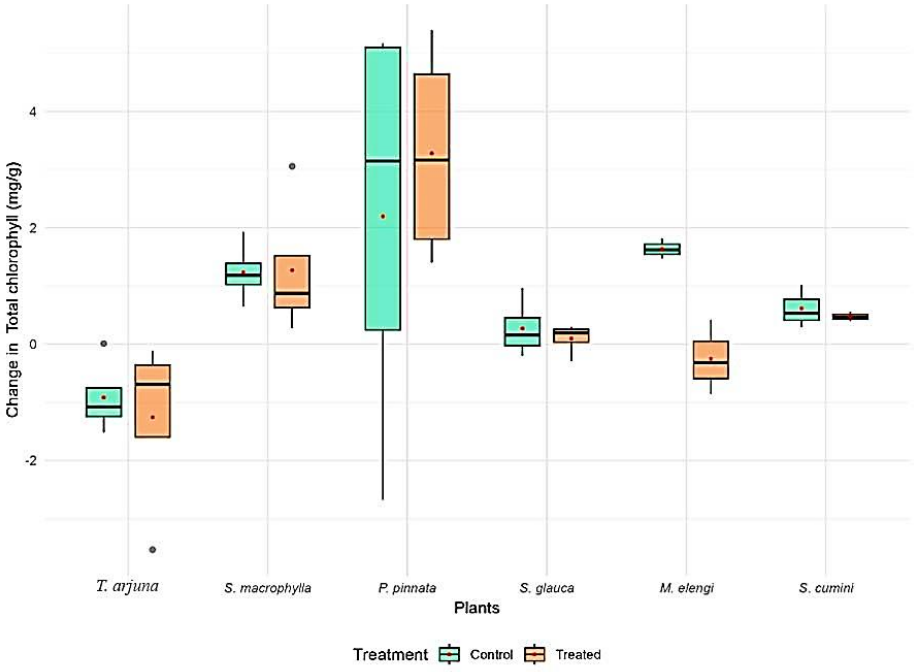


Figure 2.4.10a: Box plot representing the change in total chlorophyll

Carotenoid content in plants generally increases under enriched CO_2 conditions. The results of *S. macrophylla* and *P. pinnata* are in agreement with the current statement. *S. macrophylla* grown at TC for 15 days reported significantly higher carotenoid content (1.075±0.182) which was 129.7 % higher compared to that of the first day (0.468±0.07). An increase of 7.86 % in carotenoid content in TC is observed compared to CC. The pigment content of *P. pinnata* in the control chamber on the

15th day is (0.638±0.083) which is 3.236% higher compared to that of the initial (0.618±0.143). In *S. glauca* and *S. cumini* a similar trend was observed in carotenoid content in such a way that a 15.38% increase in carotenoid content was noticed on the final day compared to the initial day in the CC and an increase of 2.631% in TC was observed in *S. glauca* and an increase of 47.05% and 29.16 % in carotenoid content is observed in CC and TC of *S. cumini*. An increase of 151.05 % in carotenoid content was noted in CC and a decrease of 10.55% was observed in TC of *M. elengi*. The results of *T. arjuna* are contradictory to the results of other plants such as a 32.36% and 37.27% decrease in carotenoid content was noticed in CC and TC respectively **(Table 2.4.12 and Figure 2.4.11).** This might be due to the adaptability of the plant even under increased stress conditions of CO_2. 2 sample t-test revealed that there is a significant difference [t (4) = 5.458, p < 0.05] in carotenoid between the control and treatment in *M. elengi* **(Figure 2.4.11 a, Annexure 2).** Significant differences in carotenoids between the plants were reported under elevated CO_2 conditions (H = 17.46, df=5, p<0.005) (**Annexure 7**).

Table 2.4.12. Variations in Carotenoids of plants (mg g^{-1}) under elevated CO_2

Days		D1	D5	D10	D15
Terminalia arjuna					
Control	**C1**	0.84	1.33	0.79	0.7
	C2	0.58	0.82	0.57	0.28
	C3	0.52	0.57	0.71	0.42
	C4	0.68	0.6	0.85	0.37
	Avg	0.655	0.83	0.73	0.443
	SD	0.14	0.351	0.121	0.181
	% Change				-32.36
Treatment	**T1**	1.242	0.861	0.731	0.438
	T2	0.6	0.66	0.74	0.31
	T3	0.55	0.53	0.77	0.43
	T4	0.7	0.46	0.46	0.42
	Avg	0.617	0.55	0.657	0.387
	SD	0.076	0.101	0.171	0.067
	% Change				-37.27
Swietenia macrophylla					
Control	**C1**	0.45	0.83	1	1.1
	C2	0.39	0.9	1.19	0.82
	C3	0.47	0.8	0.78	1.25
	C4	0.56	0.92	0.78	1.13
	Avg	0.468	0.863	0.938	1.075
	SD	0.07	0.057	0.198	0.182
	% Change				+129.70

Treatment	**T1**	0.47	0.76	1.06	0.82
	T2	0.46	0.55	0.67	1.02
	T3	0.32	0.93	0.99	0.66
	T4	0.26	0.9	0.8	1.09
	Avg	0.378	0.785	0.88	0.898
	SD	0.104	0.173	0.178	0.195
	% Change				+137.56
Pongamia pinnata					
Control	**C1**	0.61	0.57	0.79	0.62
	C2	0.73	0.55	0.71	0.72
	C3	0.42	0.5	0.39	0.68
	C4	0.71	0.47	0.6	0.53
	Avg	0.618	0.523	0.623	0.638
	SD	0.142	0.046	0.173	0.083
	% Change				+3.236
Treatment	**T1**	0.76	0.83	0.78	0.77
	T2	0.82	0.94	0.72	0.86
	T3	0.45	0.57	0.55	0.57
	T4	0.61	0.66	0.61	0.86
	Avg	0.66	0.75	0.665	0.765
	SD	0.166	0.166	0.104	0.137
	% Change				+15.91
Simarouba glauca					
Control	**C1**	0.21	0.29	0.17	0.18
	C2	0.17	0.21	0.17	0.15
	C3	0.24	0.39	0.29	0.29
	C4	0.16	0.41	0.17	0.28
	Avg	0.195	0.325	0.2	0.225
	SD	0.037	0.093	0.06	0.07
	% Change				+15.38
Treatment	**T1**	0.18	0.22	0.21	0.21
	T2	0.15	0.21	0.18	0.17
	T3	0.16	0.34	0.24	0.19
	T4	0.27	0.27	0.15	0.21
	Avg	0.19	0.26	0.195	0.195
	SD	0.055	0.059	0.039	0.019
	% Change				+2.631
Mimusops elengi					
Control	**C1**	0.22	0.69	0.55	0.48
	C2	0.16	0.51	0.27	0.48
	C3	0.19	0.62	0.45	0.47
	Avg	0.19	0.607	0.423	0.477
	SD	0.03	0.091	0.142	0.006
	% Change				+151.05
Treatment	**T1**	0.51	0.54	0.33	0.37
	T2	0.37	0.51	0.46	0.43
	T3	0.37	0.28	0.34	0.32
	Avg	0.417	0.443	0.377	0.373
	SD	0.081	0.142	0.072	0.055
	% Change				-10.55
Syzygium cumini					
Control	**C1**	0.21	0.43	0.24	0.31
	C2	0.15	0.31	0.23	0.26

	C3	0.15	0.38	0.36	0.18
	Avg	0.17	0.373	0.277	0.25
	SD	0.035	0.06	0.072	0.066
	% Change				+47.05
Treatment	**T1**	0.24	0.28	0.14	0.31
	T2	0.25	0.33	0.21	0.33
	T3	0.23	0.39	0.16	0.29
	Avg	0.24	0.333	0.17	0.31
	SD	0.01	0.055	0.036	0.02
	% Change				+29.16

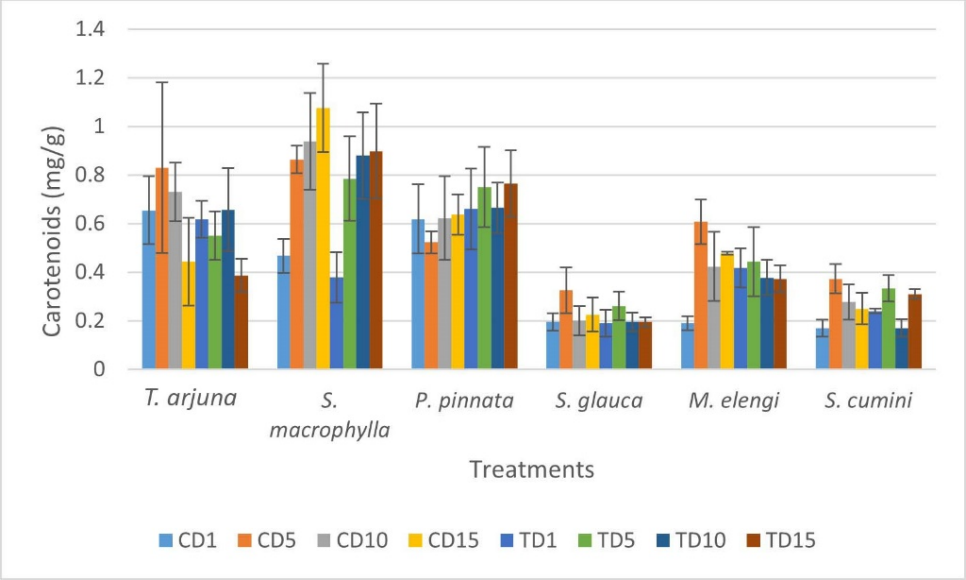


Figure 2.4.11: Effects of elevated CO_2 on carotenoids

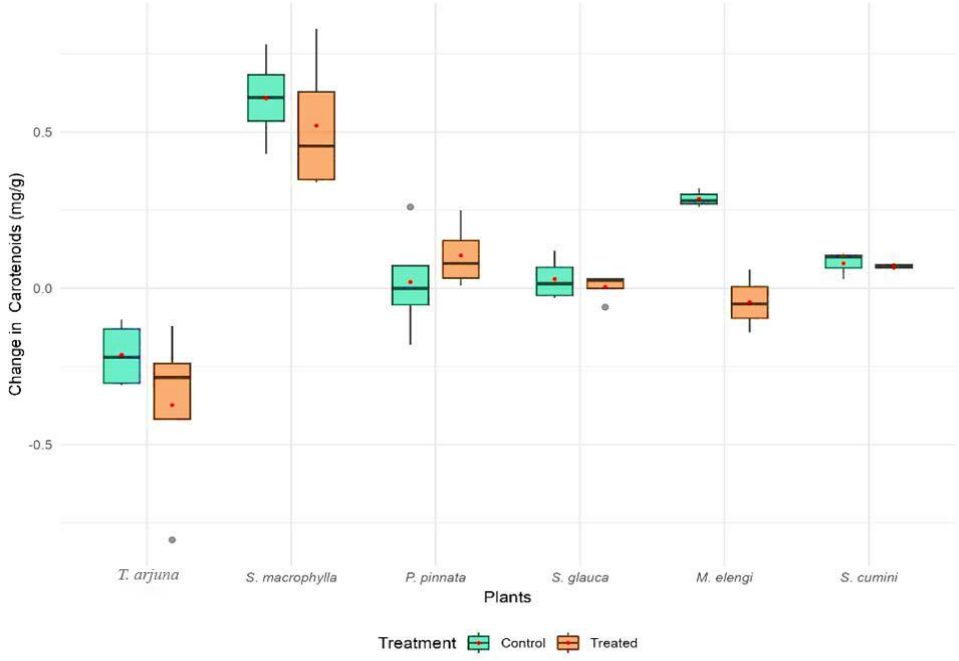


Figure 2.4.11a: Box plot representing the change in carotenoids

2.4.9.2 Plant metabolites

Carbohydrate content increased under elevated CO_2 compared to control in *T. arjuna*, *S. macrophylla*, *M. elengi*, and *S. cumini*, whereas it decreased under enriched CO_2 compared to control in *S. glauca* and *S. cumini*. *T. arjuna* grown in the TC for 15 days reported significantly higher carbohydrate content (79.04±13.08) which was 84% higher compared to that of the first day (42.96 ±6.65). Carbohydrate in the CC on the 15th day (60.55±11.75) was also 9.79% higher than that of the initial (55.19±7.902). *S. macrophylla* grown at TC after 15 days reported higher carbohydrate content (92.86±6.53) which is 38 % higher compared to the first day (67.49±11.12). In CC carbohydrate on the final day is 78.36±1.22 which is 33.58% higher compared to that of the first day (58.66 ±2.993). The increase in percentage in TC is higher compared to CC. Similarly, *M. elengi* grown in the TC after 15 days reported higher carbohydrate content (66.13± 19.58) which is 22.34 % higher compared to the first day (54.05±32.87). In CC carbohydrate on the final day is 49.82 ± 6.54 which is 1.02% higher compared to that of the first day (49.32± 4.19). In TC increase in carbohydrate is reported compared to CC. In *P. pinnata*, a 38.20% increase in carbohydrate content under elevated CO_2 is reported inside TC on the final day, compared to the first day. A slight reduction from 50.58 to 48.39 is noted on the 5th day compared to the 1st day and on the final day increased carbohydrate is noticed. *S. glauca* grown in CC after 15 days reported lower carbohydrate content (146.01±34.67) which is 6.7% lower compared to the first day (156.58±19.82). In TC carbohydrates on the final day are 137.86±26.52 which is 21.59 % lower compared to that of the first day (175.83 ±16.73). The decrease in percentage in TC is 15% lower compared to CC. The carbohydrate content of *S. cumini* increased by 49.55% in CC and 26.17% in TC on the final day compared to the initial. There is a reduction of 23.38% in carbohydrates in elevated CO_2 conditions compared to the control. The results are depicted in **Table 2.4.13** and **Figure 2.4.12.** In *T. arjuna*, significant differences in carbohydrate [$t(6) = -4.2$, $p < 0.05$] between control and treatment is reported. Similarly significant difference between control and treatment is reported in *P. pinnata* [$t(6) = -4.2$, $p < 0.05$] (**Annexure 8**). Shapiro Wilk normality test and F test were done to test the normality of data **(Figure 2.4.12 a).** Significant differences in carbohydrates between the plants were reported under elevated CO_2 conditions ($H = 13.49$, $df=5$, $p < 0.05$) (**Annexure 9**).

Table 2.4.13: Variations in Carbohydrate content (mg g^{-1}) of plants under elevated levels of CO_2

Days		D1	D5	D10	D15
Terminalia arjuna					
Control	**C1**	63.12	67.95	63.12	51.94
	C2	44.39	67.95	66.2	52.25
	C3	55.27	83.35	63.42	61
	C4	57.98	74.29	65.53	77.01
	Avg	55.19	73.385	64.568	60.55
	SD	7.902	7.285	1.528	11.75
	% Change				+9.790
Treatment	**T1**	37.15	116.57	72.48	69.46
	T2	50.13	81.24	64.33	86.07
	T3	47.11	69.16	85.47	93.92
	T4	37.45	70.97	81.84	66.74
	Avg	42.96	84.485	76.03	79.048
	SD	6.652	22.042	9.528	13.088
	% Change				+84.00
Swietenia macrophylla					
control	**C1**	62.51	62.51	54.96	78.21
	C2	59.49	49.52	64.93	79.42
	C3	56.77	70.36	57.98	79.12
	C4	55.87	49.22	51.34	76.7
	Avg	58.66	57.903	57.303	78.363
	SD	2.993	10.361	5.764	1.222
	% Change				+33.58
Treatment	**T1**	53.15	57.07	63.72	102.07
	T2	71.87	57.68	100.56	86.97
	T3	79.42	50.43	79.72	89.99
	T4	65.53	57.07	159.15	92.41
	Avg	67.493	55.563	100.788	92.86
	SD	11.12	3.434	41.73	6.531
	% Change				+38.00
Pongamia pinnata					
Control	**C1**	74.59	31.41	37.75	45.3
	C2	77.31	40.77	38.35	48.02
	C3	64.33	47.11	44.7	77.31
	C4	61.91	41.98	44.09	40.17
	Avg	69.535	40.318	41.223	52.7
	SD	7.555	6.543	3.68	16.726
	% Change				-24.21
Treatment	**T1**	44.39	39.56	55.27	59.49
	T2	48.92	77.01	112.34	97.24
	T3	54.96	41.98	48.92	64.02
	T4	54.06	35.03	94.83	58.89
	Avg	50.583	48.395	77.84	69.91
	SD	4.911	19.293	30.685	18.363
	% Change				+38.208

Simarouba glauca					
Control	**C1**	150.09	145.56	130.76	135.29
	C2	131.97	99.05	79.72	109.02
	C3	176.97	171.83	178.78	192.07
	C4	167.3	173.34	166.4	147.67
	Avg	156.583	147.445	138.915	146.013
	SD	19.82	34.693	44.404	34.677
	% Change				-6.750
Treatment	**T1**	190.26	169.12	186.33	152.51
	T2	188.14	154.62	151	163.38
	T3	154.62	118.08	113.55	132.57
	T4	170.32	119.89	110.53	102.98
	Avg	175.835	140.428	140.353	137.86
	SD	16.733	25.468	35.754	26.524
	% Change				-21.596
Mimusops elengi					
Control	**C1**	50.43	58.28	45.9	42.88
	C2	52.85	54.05	59.19	55.87
	C3	44.69	59.79	53.45	50.73
	Avg	49.323	57.373	52.847	49.827
	SD	4.191	2.975	6.666	6.542
	% Change				+1.021
Treatment	**T1**	28.69	48.32	42.88	50.73
	T2	42.28	58.28	46.5	59.49
	T3	91.2	106.9	46.5	88.18
	Avg	54.057	71.167	45.293	66.133
	SD	32.877	31.344	2.09	19.589
	% Change				+22.34
Syzygium cumini					
Control	**C1**	44.99	44.69	51.03	75.8
	C2	42.88	88.78	99.05	80.63
	C3	77.31	123.51	50.73	90.6
	Avg	55.06	85.66	66.937	82.343
	SD	19.298	39.503	27.811	7.547
	% Change				+49.55
Treatment	**T1**	49.22	46.2	50.43	75.5
	T2	59.19	54.66	60.4	69.46
	T3	53.15	77.31	64.32	58.89
	Avg	53.853	59.39	58.383	67.95
	SD	5.022	16.085	7.161	8.407
	% Change				+26.17

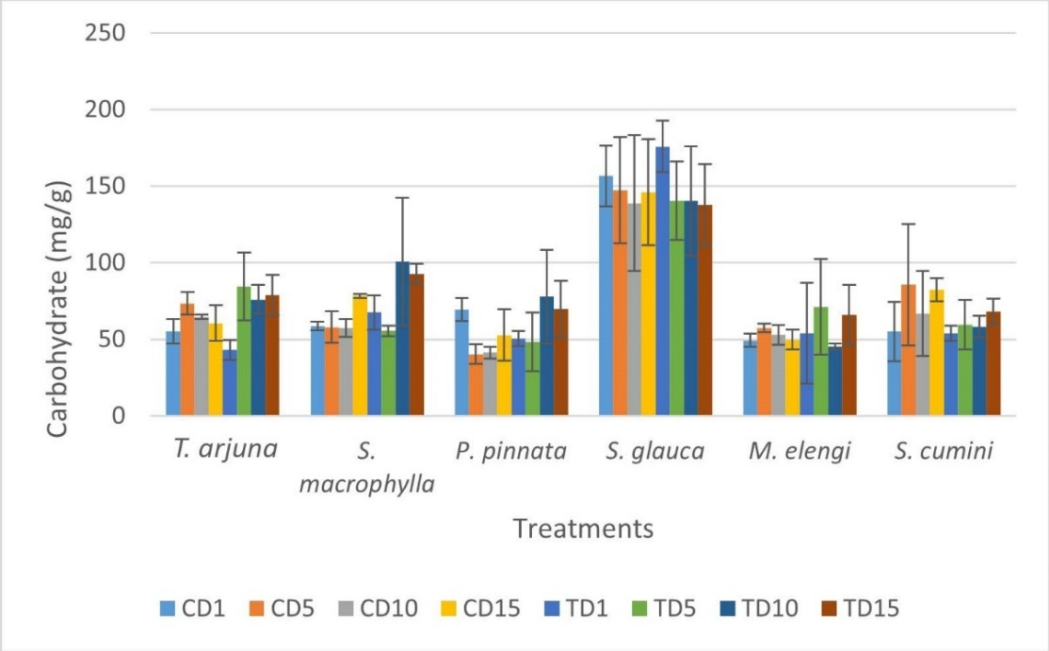


Figure 2.4.12: Effects of elevated CO_2 on carbohydrates

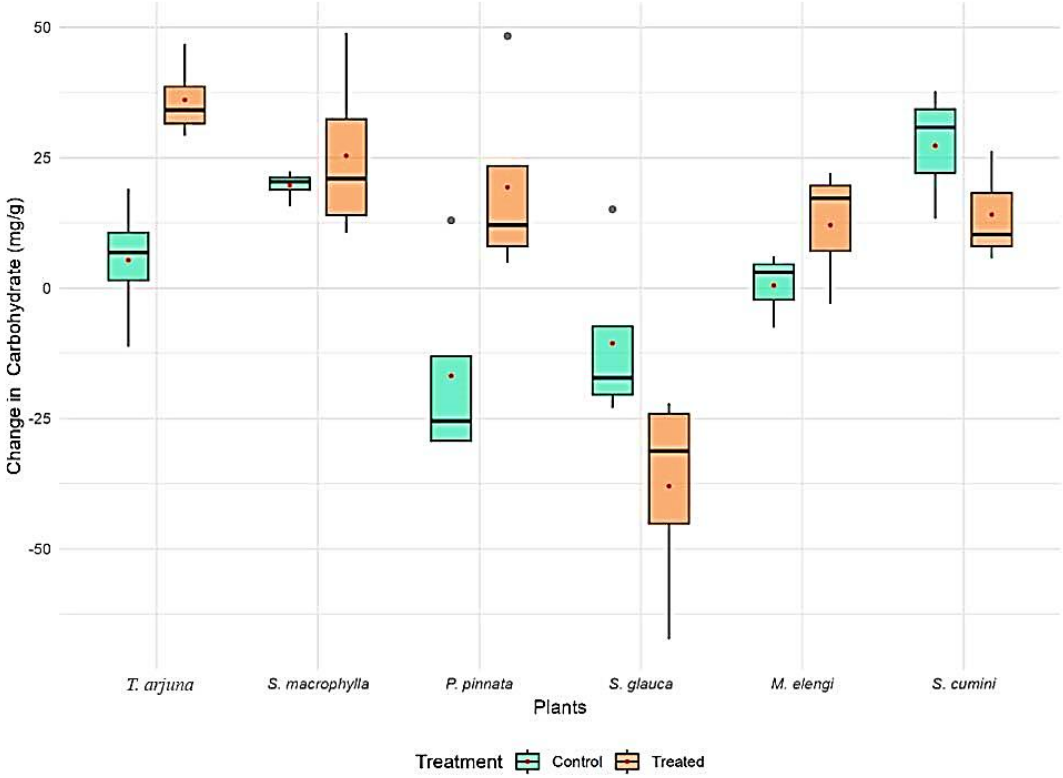


Figure 2.4.12a: Box plot representing the change in carbohydrates

T. arjuna grown at an elevated CO_2 for 15 days reported significantly higher protein content (405.63±124.05) which was 727.06 % higher compared to that of the first day (49.04±11.18). Protein content in the CC on the 15th day is (465.69±84.15)

which is 1213% higher compared to that of the initial (35.45±12.68). In *S. macrophylla* percentage increase in protein content on the final day compared to the initial day in CC and TC is 58.08% and 24.46% respectively. As far as day is concerned there is an increase in the protein content on the upcoming days in both control and treatment whereas the increase in percentage in CC is more than TC and is represented by 33.62%. Under enhanced CO_2 conditions protein content showed a slight reduction on the 5th day which then increased on the 10th and 15th day. In *P. pinnata* on comparing days, there is a slight reduction in the protein content in both CC and TC, which might be due to the decrease in metabolite when the days progressed. In TC there is a slight reduction of 74.21% in the protein content compared to CC (72.87%). In *S. glauca* and *M. elengi* protein content showed a declining trend in both CC and TC as the days progressed. In *S. glauca* protein content decreased by 6.496% in CC and 9.745% in TC. A reduction of 3.25% in TC compared to CC is noticed whereas in *M. elengi* protein content decreased by 11% in CC and 23.56% in TC. A reduction of 12.56% in TC compared to CC is noticed. *S. cumini* grown at an elevated CO_2 condition for 15 days reported significantly higher protein content (900.13±176.69) which is 31.74% higher compared to that of the first day (683.24±59.96). Protein content in the control chamber on the 15th day is 1015.37 (±193.58) which is 241.03% higher than that of the initial (297.73±185.82) (**Table 2.4.14 and Figure 2.4.13**). A 2-sample t-test was conducted and found that there is no significant difference between the treatments in any plants (**Annexure 8**). From the results of the F test, it is understood that the data is normally distributed which is represented in the box plot **(Figure 2.4.13 a).** Significant differences in protein between the plants are reported under elevated CO_2 conditions ($H = 16.911$ $df= 5$, $p<0.005$) (**Annexure 9**)

Table 2.4.14: Variations in Protein (mg g^{-1}) of plants under elevated levels of CO_2

Days		**D1**	**D5**	**D10**	**D15**
Terminalia arjuna					
Control	**C1**	28.79	116.88	481.25	461.53
	C2	50.51	145.68	753.83	358.97
	C3	21.86	86.85	586.74	564.1
	C4	40.65	78.84	667.59	478.17
	Avg	35.453	107.063	622.353	465.693
	SD	12.687	30.511	116.205	84.156
	% Change				+1213

Treatment	**T1**	45.89	81.77	584.43	458.3
	T2	41.42	144.76	677.6	223.14
	T3	65.6	131.82	574.42	499.42
	T4	43.27	108.57	628.32	441.67
	Avg	49.045	116.73	616.193	405.633
	SD	11.188	27.702	47.158	124.059
	% Change				+727.06
Swietenia macrophylla					
Control	**C1**	312.31	289.21	491.1	585.2
	C2	337.72	262.41	257.33	590.59
	C3	297.06	100.71	222.22	495.11
	C4	541.92	559.94	475.86	682.99
	Avg	372.253	303.068	361.628	588.473
	SD	114.348	190.418	141.568	76.736
	% Change				+58.08
Treatment	**T1**	416.72	421.8	393.16	623.7
	T2	540.07	516.97	553.93	679.91
	T3	541	306.76	303.07	520.52
	T4	461.53	286.9	350.65	614.46
	Avg	489.83	383.108	400.203	609.648
	SD	61.342	107.24	108.891	66.084
	% Change				+24.46
Pongamia pinnata					
Control	**C1**	269.5	262.41	273.19	84.08
	C2	323.09	405.32	363.13	61.29
	C3	319.7	362.51	374.52	128.13
	C4	335.72	431.2	297.52	64.99
	Avg	312.003	365.36	327.09	84.623
	SD	29.161	74.249	49.424	30.676
	% Change				-72.87
Treatment	**T1**	196.19	222.68	226.68	70.53
	T2	299.99	313.23	304.61	100.1
	T3	386.84	401.63	411.79	61.29
	T4	321.86	346.5	259.64	78.85
	Avg	301.22	321.01	300.68	77.693
	SD	79.14	75.008	80.666	16.571
	% Change				-74.21
		D1	**D5**	**D10**	**D15**
Simarouba glauca					
Control	**C1**	870.1	703.01	333.41	836.22
	C2	732.65	729.19	203.28	722.26
	C3	732.27	685.3	455.84	595.21
	C4	627.93	436.59	424.27	616.77
	Avg	740.738	638.523	354.2	692.615
	SD	99.327	135.824	113.21	110.667
	% Change				-6.496
Treatment	**T1**	617.92	574.42	485.1	608.3
	T2	755.37	722.26	798.41	663.74
	T3	528.99	439.67	512.05	466.62
	T4	523.21	351.89	468.16	450.45
	Avg	606.373	522.06	565.93	547.278
	SD	108.378	161.831	156.037	105.148
	% Change				-9.745

Mimusops elengi					
Control	**C1**	214.06	174.79	234.85	200.2
	C2	338.8	354.97	254.1	249.48
	C3	237.93	302.61	277.2	254.1
	Avg	263.597	277.457	255.383	234.593
	SD	66.213	92.686	21.204	29.875
	% Change				-11.00
Treatment	**T1**	300.3	238.7	290.29	228.69
	T2	368.06	251.02	317.24	221.76
	T3	217.14	227.92	204.82	226.38
	Avg	295.167	239.213	270.783	225.61
	SD	75.591	11.559	58.694	3.529
	% Change				-23.563
Syzygium cumini					
Control	**C1**	508.2	210.98	614.46	954.8
	C2	156.31	418.11	636.79	859.32
	C3	228.69	243.32	746.9	1232
	Avg	297.733	290.803	666.05	1015.373
	SD	185.828	111.43	70.903	193.583
	% Change				+241.034
Treatment	**T1**	659.12	595.21	918.61	877.03
	T2	751.52	169.4	1099.56	736.12
	T3	639.1	308.77	938.63	1087.24
	Avg	683.247	357.793	985.6	900.13
	SD	59.968	217.097	99.199	176.696
	% Change				+31.74

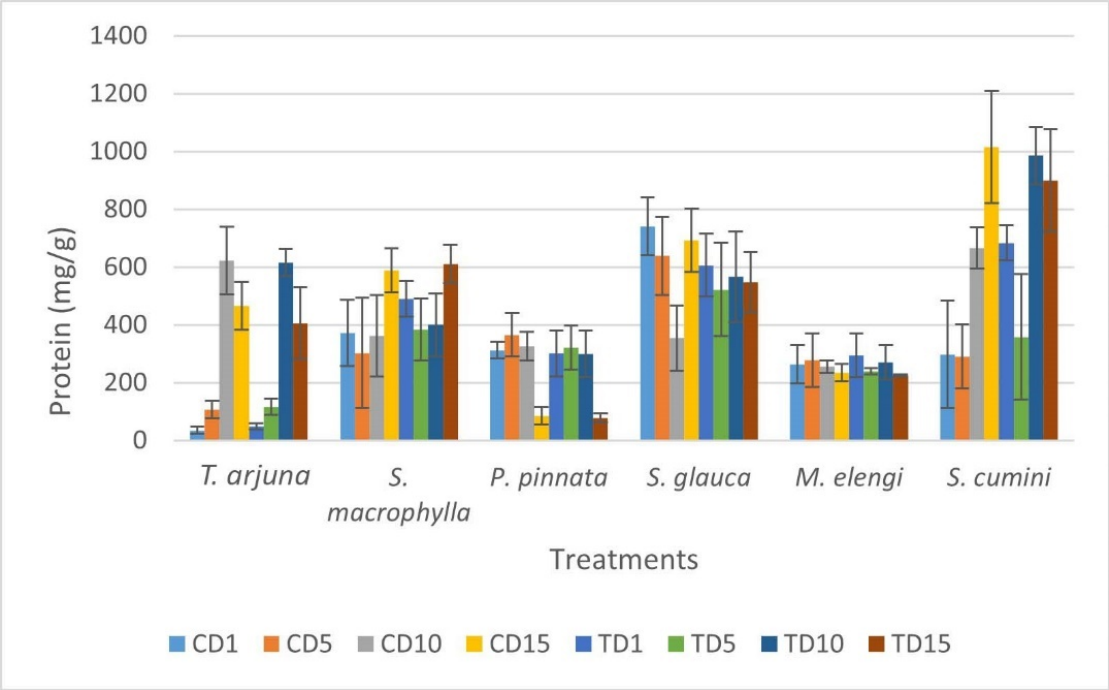


Figure 2.4.13: Effects of elevated CO_2 on protein

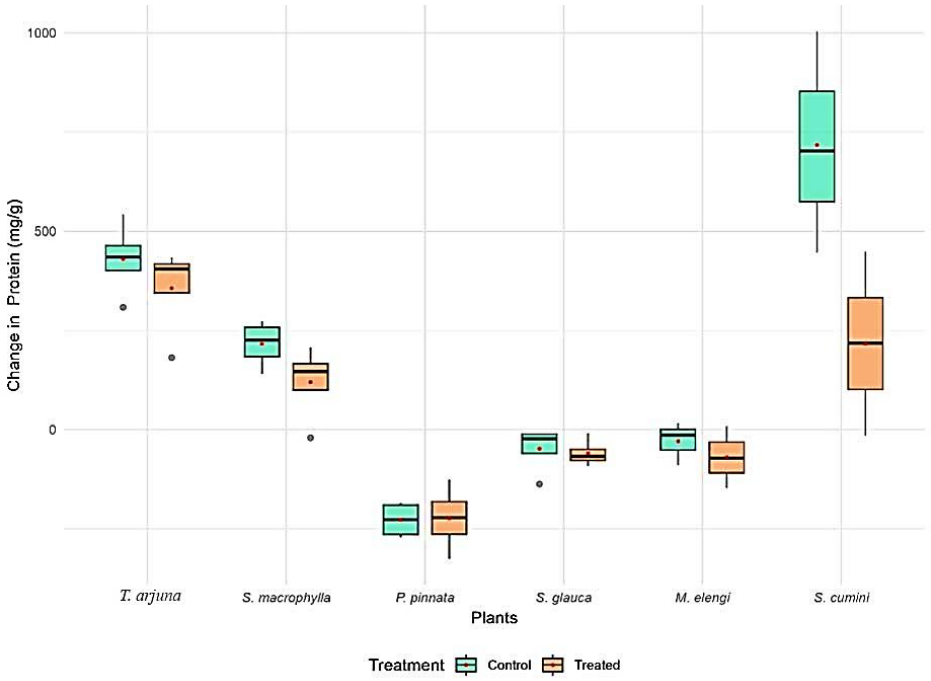


Figure 2.4.13a: Box plot representing the change in protein

S. macrophylla and *P. pinnata* grown in the TC are reported to have significantly higher phenol content. In the case of *T. arjuna*, in TC, an increase of 150.1% was noted on the final day compared to the initial day. In *S. glauca*, *M. elengi,* and *S. cumini* there is a reduction of phenol content by 22.52 %, 37.21 %, and 2.604 % respectively in TC. With the progressing of days, there is a reduction of phenol content in TC, compared to CC in *T. arjuna*, *S. glauca*, *M. elengi,* and *S. cumini* **(Table 2.4.15 and Figure 2.4.14).** Results of 2 sample t-tests in *S. macrophylla* indicate that there is a significant difference in phenol between the control and treatment where [t (6) = -7.693, $p < 0.05$] **(Figure 2.4.14 a**, **Annexure 8).** Significant differences in phenol among the plants were reported under elevated CO_2 conditions (H =16.068, df=5, $p < 0.01$) (**Annexure 9**).

Table 2.4.15. Variations in Phenol (mg g^{-1}) of plants under elevated levels of CO_2

Days		D1	D5	D10	D15
Terminalia arjuna					
Control	**C1**	186.15	311.37	369.15	533.79
	C2	171.38	388	235.61	531.03
	C3	143.72	430.99	226.23	788.28
	C4	198.11	480.7	533.79	533.79
	Avg	174.84	402.765	341.195	596.723
	SD	23.451	71.744	144.036	127.712
	% Change				+241.2

Treatment	**T1**	147.65	289.6	476.2	505.31
	T2	252.12	896.22	393.43	511.74
	T3	264.08	326.7	491.1	794.71
	T4	263.15	336.79	584.35	507.14
	Avg	231.75	462.328	486.27	579.725
	SD	56.329	289.972	78.238	143.349
	% Change				+150.1
Swietenia macrophylla					
Control	**C1**	268.34	324.5	368.33	380.8
	C2	345.23	367.8	386.4	380.56
	C3	289.45	290.56	345.86	380.37
	C4	345.9	369.2	349	425.25
	Avg	312.23	338.015	362.398	391.745
	SD	39.446	37.834	18.835	22.337
	% Change				+25.46
Treatment	**T1**	235.25	356.36	415.86	480.56
	T2	260.45	370.58	534	580.56
	T3	267.76	267.76	490.45	516.67
	T4	295.57	295.57	423.62	545.87
	Avg	264.758	322.568	465.983	530.915
	SD	24.817	48.921	56.368	42.532
	% Change				+100.5
Pongamia pinnata					
Control	**C1**	57.79	34.08	150.55	169.4
	C2	106.1	47.41	205.08	198.76
	C3	348.82	38.52	264.06	189.34
	C4	248.95	42.08	269.1	196.78
	Avg	190.415	40.523	222.198	188.57
	SD	133.188	5.639	55.913	13.408
	% Change				-0.968
Treatment	**T1**	43.56	21.33	163.59	180.2
	T2	146.11	24.59	136.03	166.2
	T3	187.89	56.01	347.34	190.36
	T4	82.09	26.96	131.58	173.4
	Avg	114.913	32.223	194.635	177.54
	SD	64.466	16.025	102.783	10.282
	% Change				+54.49
Simarouba glauca					
Control	**C1**	392.49	364.45	315.06	324.4
	C2	406.73	270.11	364.45	277.68
	C3	393.82	262.55	308.35	261.66
	C4	491.28	245.19	218.9	324.4
	Avg	421.08	285.575	301.69	297.035
	SD	47.239	53.608	60.597	32.268
	% Change				-29.45
Treatment	**T1**	296.8	259.88	365.79	423.19
	T2	347.1	350.21	426.31	208.26
	T3	635.9	25365	234.96	284.8
	T4	130.38	163.31	267.89	176.22
	Avg	352.545	6534.6	323.738	273.118
	SD	210.384	12553.83	88.11	109.93
	% Change				-22.52

Mimusops elengi					
Control	**C1**	223.39	318.17	348.43	178
	C2	312.39	347.54	293.7	191.79
	C3	201.14	398.72	259.88	125.04
	Avg	245.64	354.81	300.67	164.943
	SD	58.868	40.764	44.685	35.238
	% Change				-32.85
Treatment	**T1**	346.65	279.46	236.29	165.98
	T2	300.82	374.69	379.14	192.68
	T3	274.56	384.48	394.27	220.27
	Avg	307.343	346.21	336.567	192.977
	SD	36.485	58.014	87.171	27.146
	% Change				-37.21
Syzygium cumini					
Control	**C1**	197.13	154.41	174.88	217.6
	C2	102.35	293.7	237.63	217.16
	C3	391.15	323.51	312.83	618.55
	Avg	230.21	257.207	241.78	351.103
	SD	147.214	90.264	69.069	231.616
	% Change				+52.51
Treatment	**T1**	371.13	190.9	391.6	359.11
	T2	437.43	227.39	347.54	256.76
	T3	199.8	225.61	399.16	366.23
	Avg	336.12	214.633	379.433	327.367
	SD	122.623	20.573	27.878	61.251
	% Change				-2.604

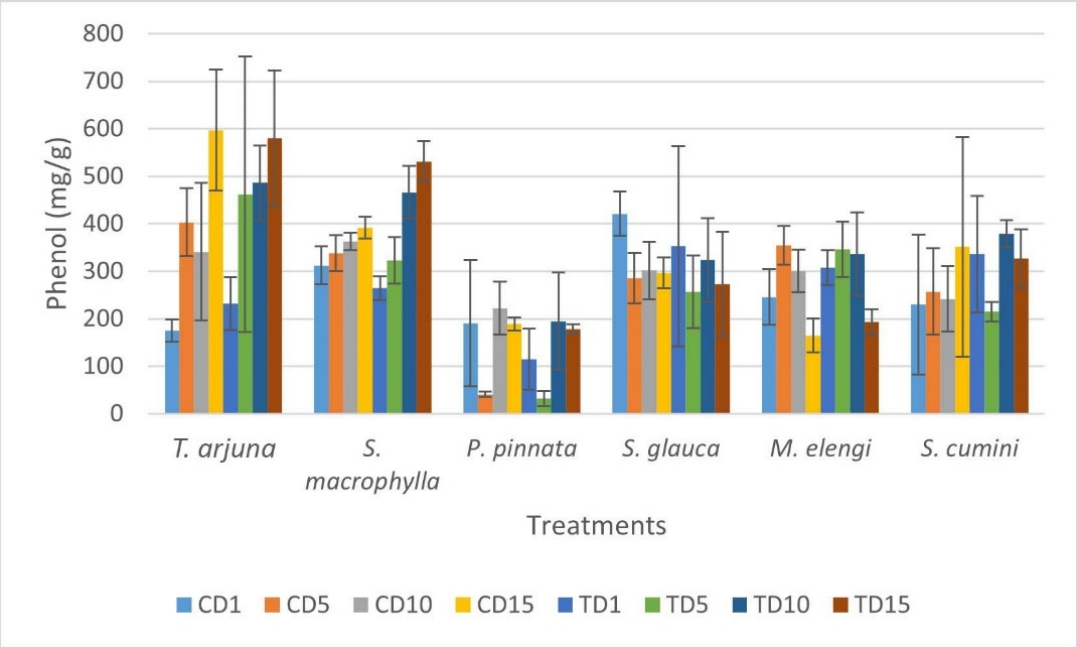


Figure 2.4.14: Effects of elevated CO_2 on phenol

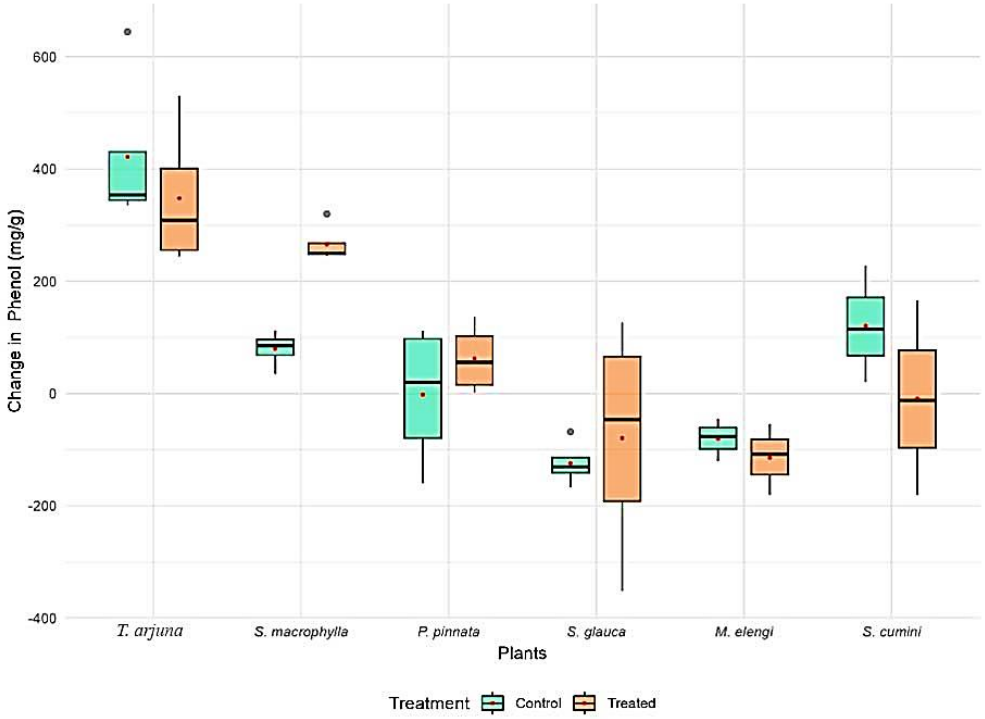


Figure 2.4.14a: Box plot representing the change in phenol

2.4.10 Minerals

Calcium content in *S. macrophylla* increased by 33.38 % under elevated CO_2 conditions and 8.201% in control compared to the first day. Calcium content decreased under enhanced CO_2 conditions in all other plants compared to the control. The reduction in calcium content is more prominent in *T. arjuna* and *S. cumini*. In *T. arjuna* calcium content decreased from 1.803±0.24 to 1.35±0.129. In *S. cumini* it decreased from 2.414± 0.008 to 1.817± 0.185. (**Figure 2.4.15**). In *T. arjuna*, significant differences in calcium [$t(6) = 6.397$, $p < 0.05$] between the control and treatments were reported. Similarly in *S. cumini* significant differences [$t(4) = 2.619$, $p < 0.05$] between the control and treatment were recorded (**Figure 2.4.15 a**, **Annexure 10**).

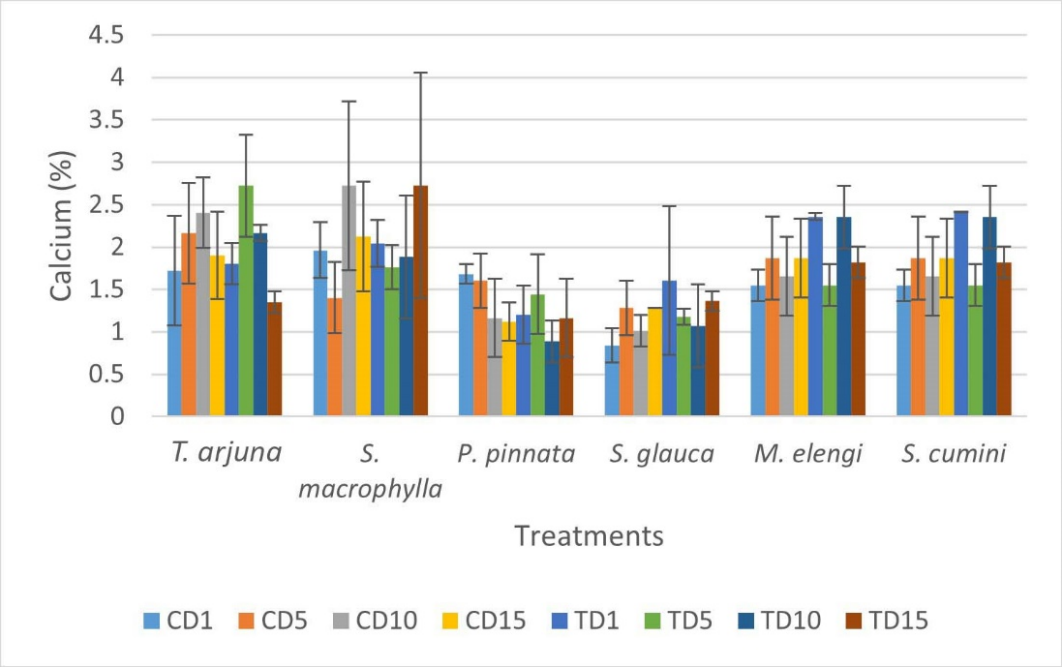


Figure 2.4.15: Effects of elevated CO_2 on calcium

The magnesium content of *P. pinnata*, *M. elengi,* and *S. cumini* increased under enriched CO_2 conditions compared to the control. *P. pinnata* grown at TC for 15 days reported significantly higher magnesium content (0.341±0.258) which was 131.9 % higher compared to that of the first day (0.147±0.04). A 61.59 % increase in magnesium is noticed in TC compared to CC. *M. elengi* grown in the TC for 15 days reported significantly higher magnesium content (0.552±0.46) which was 475 % higher compared to that of the first day (0.096±0.001). Similarly, *S. cumini* grown in TC for 15 days reported higher magnesium content (0.548±0.462) which was 515.7% higher compared to the first day (0.089±0.009). In *S. glauca* 81.01% increase in magnesium content is reported compared to that of the first day. In TC there is an increase of 23.24% in magnesium content on the final day compared to the initial day. In *T. arjuna* and *S. macrophylla* magnesium content decreased under enriched CO_2 conditions compared to the control **(Figure 2.4.16)**. 2 sample t-tests revealed that there is no significant difference between the treatments in magnesium content of all plants since $P > 0.05$. From the results of the Shapiro-Wilk normality test and F test, the data is non-normal in the distribution in *S. macrophylla* and *S. glauca* since $p < 0.05$ **(Figure 2.4.16 a).** Significant differences in magnesium between the plants were reported under elevated CO_2 conditions ($H = 10.984$, $df = 5$, $p < 0.05$) (**Annexure 11**).

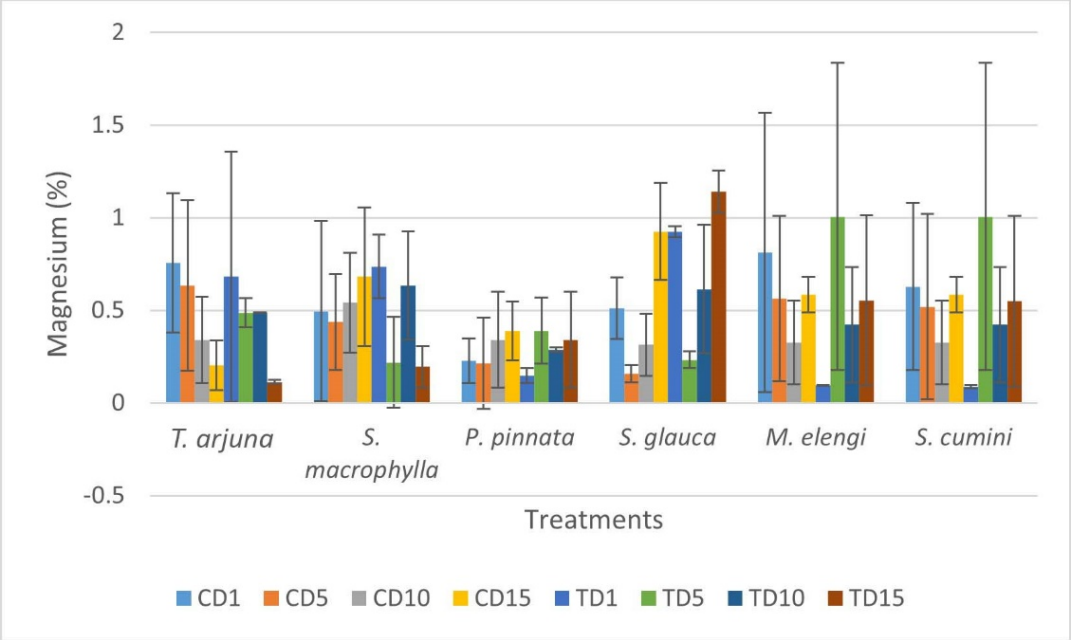


Figure 2.4.16: Effects of elevated CO_2 on Magnesium

The sodium content of all the plants decreased under enhanced CO_2 conditions compared to the control. In the case of *S. cumini*, in TC, as far as days are concerned, there is an increase of 93.04% sodium content on the final day compared to the first day followed by an increase of 77.93% in *S. macrophylla* followed by an increase of 45.12% in *P. pinnata.* In *S. cumini* and *P. pinnata* there is an increasing trend of sodium towards the 10^{th} day of CO_2 treatment, followed by a decline towards the 15^{th} day of CO_2 treatment. Similarly, in *S. macrophylla* sodium content increased on the 10^{th} day of CO_2 treatment which drastically reduced on the 15^{th} day of CO_2 treatment (**Figure 2.4.17**). Two sample t-tests were done to test the differences in sodium between the control and treatments. In *S. macrophylla* significant difference was observed [t (6) = 2.283, $p < 0.05$] between control (0.233±0.005) and treatment (0.258±0.049). Similarly, in *S. glauca* significant difference [t (6) = 1.926, $p < 0.05$] was observed between control (0.129 ± 0.009) and treatment (0.126 ± 0.004). Also with *S. cumini* significant difference was observed [t (4) = 4.414, $p < 0.05$] between control (0.113±0.006) and treatment (0.115 ± 0.013) (**Figure 2.4.17 a**, **Annexure 10**). Significant differences in sodium between the plants were also reported under elevated CO_2 conditions (H =18.291, df=5, p<0.005) (**Annexure 11**).

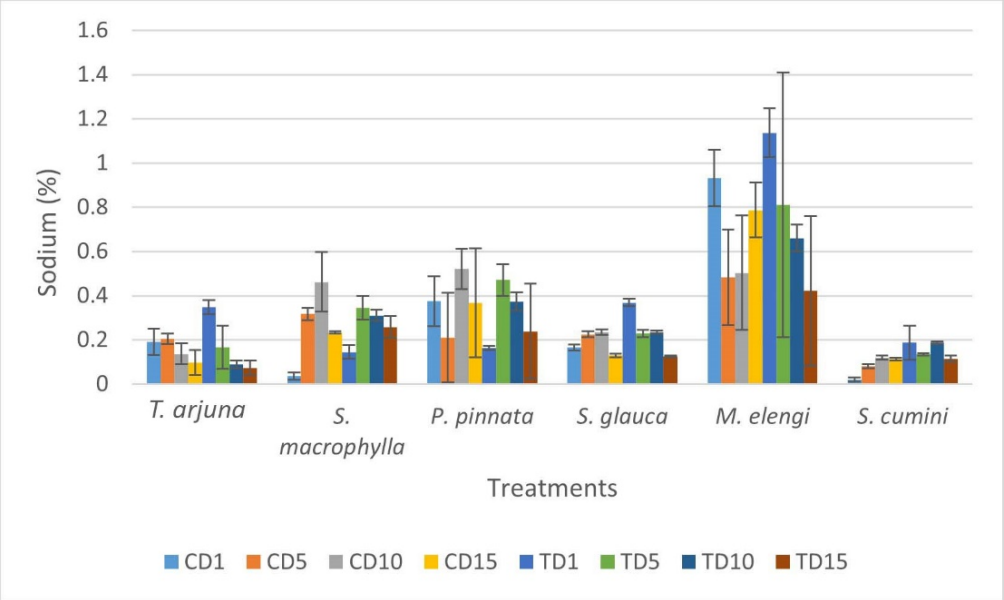


Figure 2.4.17: Effects of elevated CO_2 on sodium

The potassium content of *S. macrophylla* and *M. elengi* increased under enriched CO_2 conditions compared to the control. In the CC of *S. macrophylla,* a 205.6% increase in potassium is observed compared to an increase of 332.6% in TC. In *M. elengi* potassium content decreased from 1.913±0.247 to 0.607±0.523. An increase of 111.7% of potassium in *M. elengi* is observed in TC on the 15th day of CO_2 treatment compared to the first day (**Figure 2.4.18**). There is a reduction in potassium content under elevated CO_2 conditions in *T. arjuna*, *P. pinnata*, *S. glauca,* and *S. cumini* **(Table 2.4.16)**. The reduction in potassium content under elevated CO_2 in *P. pinnata* [t (6) = 3.364, $p < 0.05$], *S. glauca* [t (6) = 3.288, $p < 0.05$], *S. cumini* [t (4) = 727, $p < 0.05$] is statistically significant **(Figure 2.4.18 a**, **Annexure 10**). Significant differences in potassium between the plants were reported under elevated CO_2 conditions (H = 20.09, df= 5, $p < 0.05$) (**Annexure 11**).

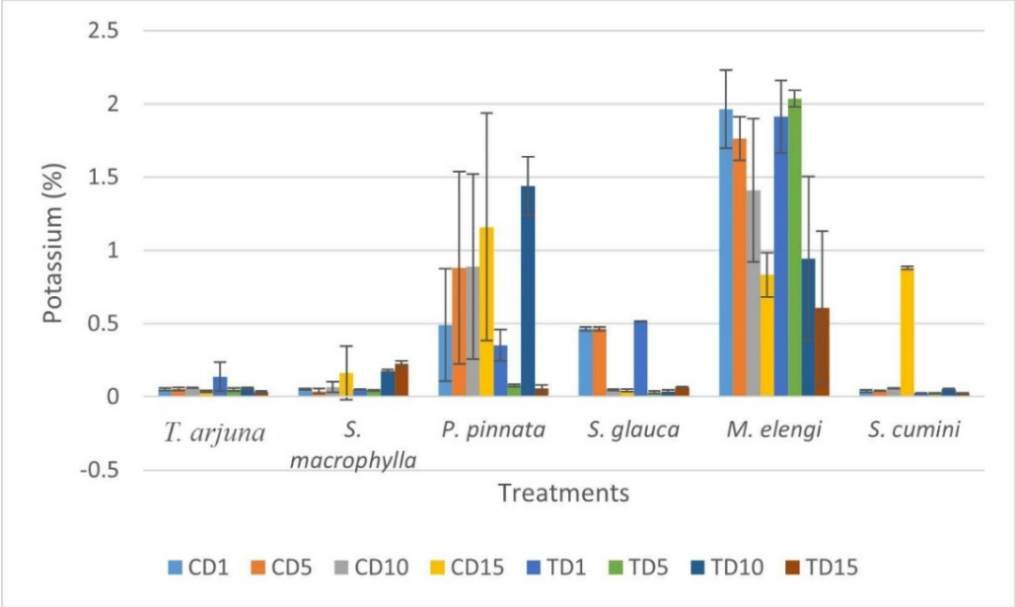


Figure 2.4.18: Effects of elevated CO_2 on potassium

Table 2.4.16: Effect of elevated CO_2 on mineral contents of plants.

	Days	Ca (%)	Mg (%)	Na (%)	K (%)
Terminalia arjuna					
CONTROL	1DOT	1.723±0.646	0.755±0.376	0.19±0.059	0.051±0.008
	5DOT	2.164±0.593	0.633±0.46	0.205±0.023	0.055±0.01
	10DOT	2.406±0.415	0.34±0.232	0.137±0.048	0.062±0.003
	15DOT	1.898±0.515	0.203±0.135	0.098±0.056	0.038±0.005
TREATMENT	1DOT	1.803±0.24	0.682±0.675	0.348±0.032	0.137±0.099
	5DOT	2.724±0.601	0.487±0.08	0.165±0.098	0.05±0.012
	10DOT	2.164±0.092	0.487±0	0.089±0.018	0.059±0.006
	15DOT	1.35±0.129	0.113±0.01	0.073±0.033	0.035±0.006
Swietenia macrophylla					
CONTROL	1DOT	1.963±0.33	0.495±0.23	0.051±0.046	0.053±0.003
	5DOT	1.402±0.422	0.436±0.258	0.317±0.027	0.039±0.017
	10DOT	2.722±0.994	1.316±1.02	0.462±0.134	0.152±0.10
	15DOT	2.124±0.646	0.681±0.373	0.233±0.005	0.162±0.11
TREATMENT	1DOT	2.043±0.274	0.736±0.171	0.145±0.031	0.052±0.002
	5DOT	1.761±0.262	0.219±0.20	0.345±0.053	0.043±0.003
	10DOT	1.882±0.723	0.633±0.292	0.31±0.025	0.177±0.009
	15DOT	2.725±1.133	0.195±0.113	0.258±0.049	0.225±0.021
Pongamia pinnata					
CONTROL	1DOT	1.683±0.113	0.244±0.207	0.399±0.125	0.564±0.448
	5DOT	1.603±0.321	0.26±0.12	0.323±0.231	1.033±0.908
	10DOT	1.162±0.46	0.341±0.258	0.52±0.09	0.888±0.632
	15DOT	1.122±0.227	0.39±0.159	0.31±0.309	0.82±0.13

TREATMENT	1DOT	1.202±0.34	0.146±0.069	0.169±0.001	0.328±0.144
	5DOT	1.443±0.472	0.39±0.178	0.485±0.082	0.563±0.125
	10DOT	0.855±0.245	0.292±0	0.37±0.05	1.41±0.234
	15DOT	1.162±0.46	0.341±0.258	0.183±0.117	0.377±0.155
Simarouba glauca					
CONTROL	1DOT	0.842±0.202	0.511±0.166	0.165±0.013	0.465±0.013
	5DOT	1.282±0.321	0.162±0.056	0.225±0.013	0.465±0.013
	10DOT	1.015±0.185	0.325±0.203	0.235±0.013	0.048±0.006
	15DOT	1.282±0	1±0.424	0.129±0.009	0.043±0.009
TREATMENT	1DOT	1.603±0.878	0.925±0.399	0.368±0.017	0.515±0.003
	5DOT	1.176±0.093	0.227±0.056	0.228±0.017	0.031±0.01
	10DOT	1.069±0.49	0.584±0.542	0.235±0.006	0.039±0.009
	15DOT	1.363±0.113	1.05±0.071	0.126±0.004	0.065±0.004
Mimusops elengi					
CONTROL	1DOT	1.55±0.185	0.779±0.797	0.932±0.128	1.963±0.266
	5DOT	1.87±0.49	0.519±0.5	0.525±0.29	1.785±0.205
	10DOT	1.656±0.463	0.325±0.225	0.503±0.259	1.41±0.49
	15DOT	1.87±0.463	0.584±0.097	0.787±0.124	0.4±0.563
TREATMENT	1DOT	2.358±0.043	0.096±0.001	1.137±0.111	1.913±0.247
	5DOT	1.55±0.245	1.006±0.828	0.66±0.764	2.053±0.067
	10DOT	2.351±0.37	0.422±0.313	0.66±0.061	0.943±0.561
	15DOT	1.817±0.185	0.552±0.46	0.421±0.338	0.583±0.556
Syzygium cumini					
CONTROL	1DOT	1.55±0.185	0.779±0.797	0.02±0.01	0.04±0.01
	5DOT	1.87±0.49	0.519±0.5	0.08±0.01	0.042±0.003
	10DOT	1.656±0.463	0.325±0.225	0.12±0.01	0.059±0.001
	15DOT	1.87±0.463	0.584±0.097	0.113±0.006	0.88±0.01
TREATMENT	1DOT	1.55±0.185	0.089±0.009	0.187±0.078	0.024±0.001
	5DOT	1.55±0.245	1.006±0.828	0.135±0.005	0.024±0.001
	10DOT	2.351±0.37	0.422±0.313	0.187±0.006	0.056±0.002
	15DOT	1.817±0.185	0.552±0.46	0.115±0.013	0.024±0.001

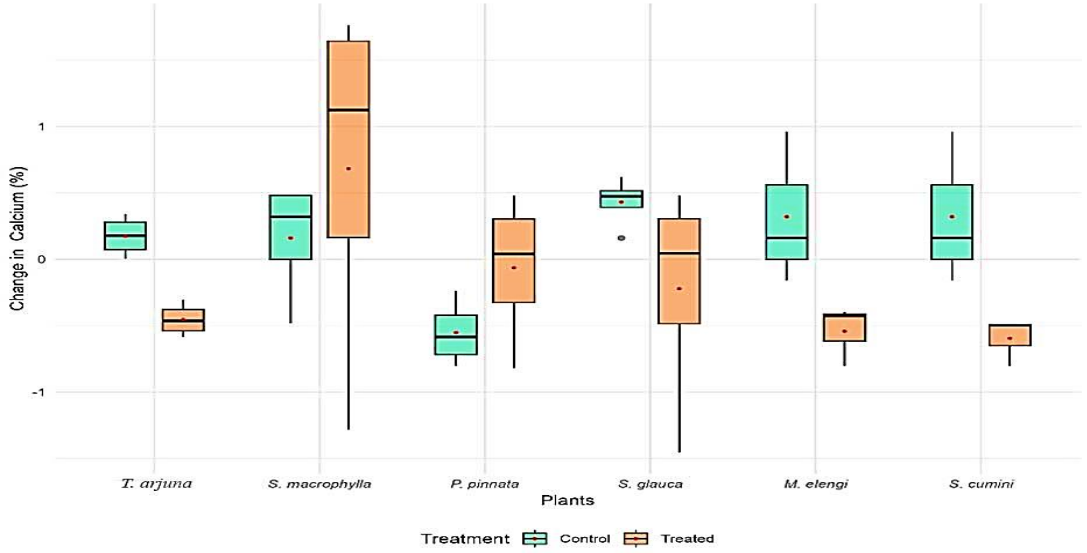


Figure 2.4.15a: Box plot representing the change in calcium

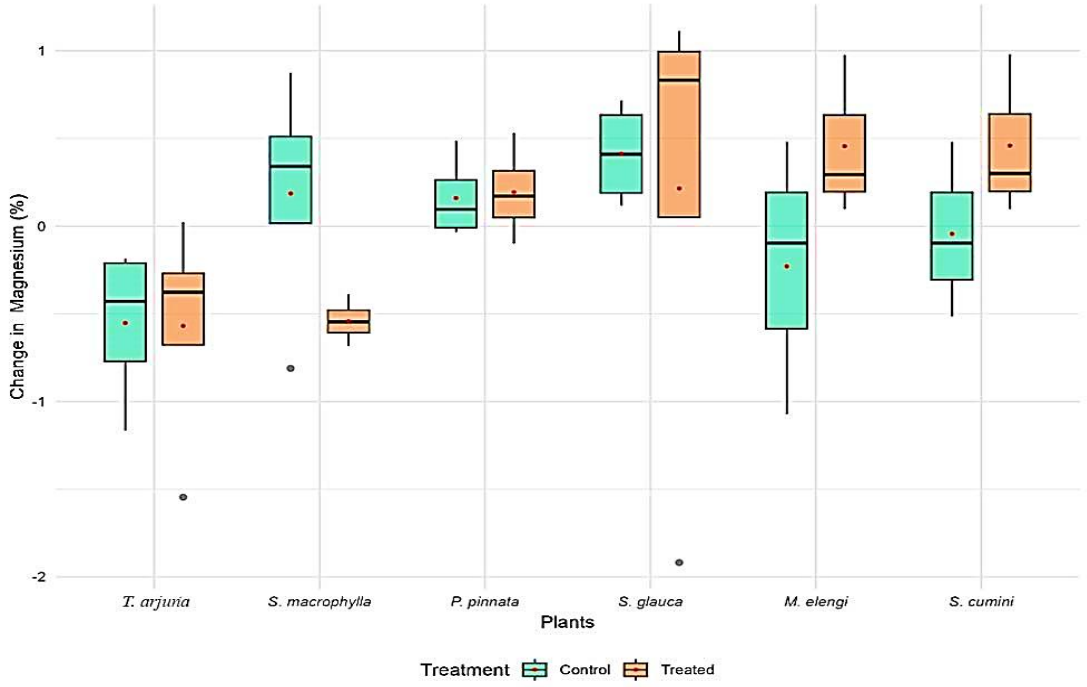


Figure 2.4.16a: Box plot representing the change in magnesium

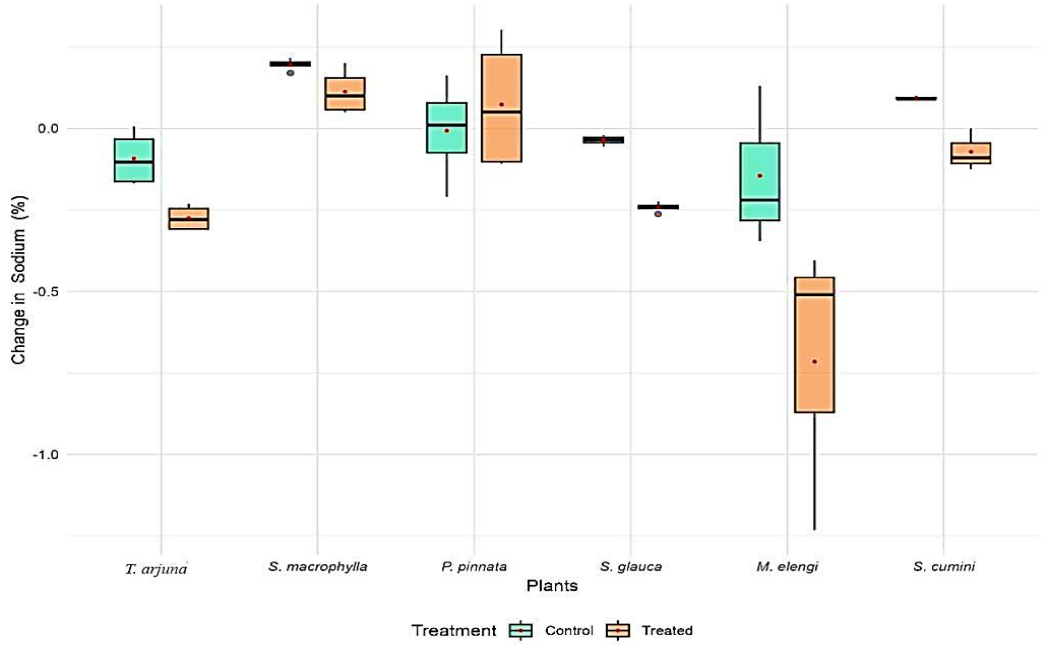


Figure 2.4.17a: Box plot representing the change in sodium

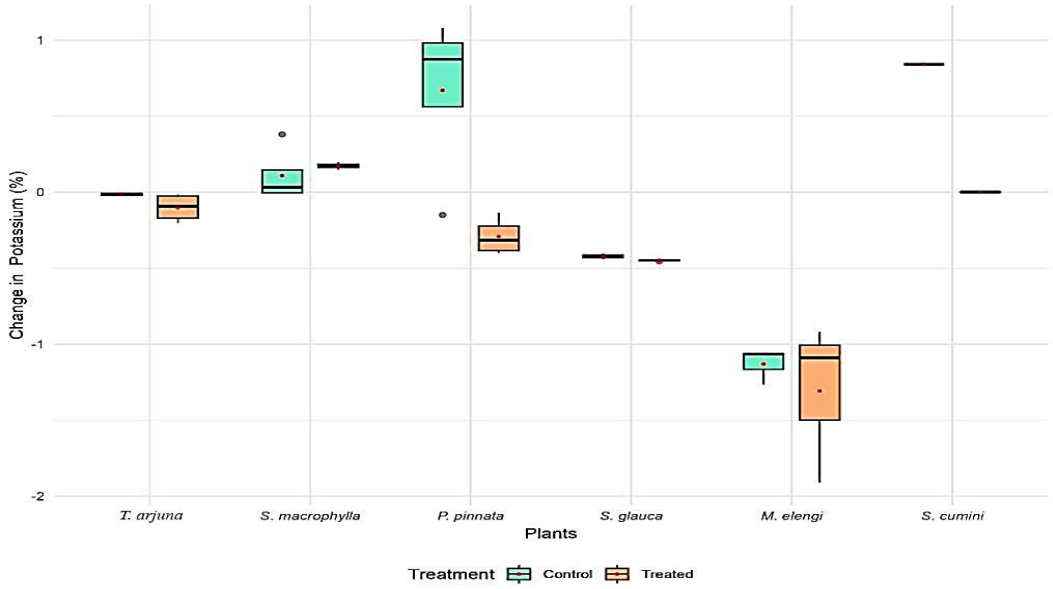


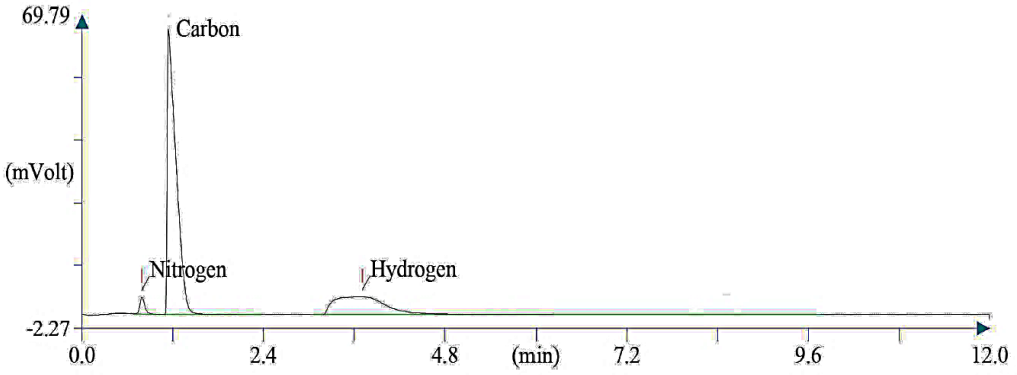
Figure 2.4.18a: Box plot representing the change in potassium

2.4.11 Plant macronutrients

Plant nitrogen content increased under elevated CO_2 conditions in *S. macrophylla* and *M. elengi*, whereas it decreased under enriched CO_2 conditions in *T. arjuna*, *P. pinnata*, *S. glauca,* and *S. cumini*. Nitrogen dilution in certain plants may have occurred due to the increased photo assimilation of carbon. Plant carbon content increased under enhanced CO_2 conditions in *S. macrophylla*, and *S. glauca* whereas it decreased in *T. arjuna*, *P. pinnata*, *M. elengi,* and *S. cumini* **(Table 2.4.16)**. **Figure 2.4.19 a to x** represents the peaks obtained for the plants after CHNS analysis in both the control and CO_2 treatments.

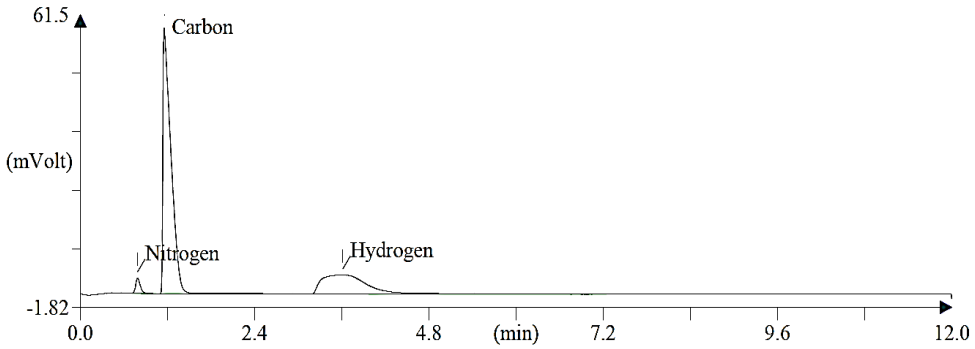
Table 2.4.17: Effects of elevated CO_2 on plant nitrogen and carbon

Nitrogen (%)	Control			Treated		
Plants	CD1	CD15	% Change	TD1	TD15	% Change
Terminalia arjuna	2.5	2.38	-4.80	2.43	2.34	-3.70
Swietenia macrophylla	2.04	1.89	-7.35	1.81	1.98	+9.39
Pongamia pinnata	3.03	3.18	+4.95	3.58	3.46	-3.35
Simarouba glauca	0.85	1.05	+23.52	1.11	0.9	-18.92
Mimusops elengi	2.31	2.14	-7.36	2.05	2.16	+5.37
Syzygium cumini	1.7	2.12	+24.70	1.26	1.24	-1.59
Carbon (%)						
Terminalia arjuna	43.42	41.7	-3.961	43.74	42.42	-3.02
Swietenia macrophylla	44.43	48.59	+9.363	44.47	48.25	+8.50
Pongamia pinnata	44.37	45.54	+2.637	44.15	43.5	-1.47
Simarouba glauca	44.76	44.76	0.000	44.65	45.19	+1.21
Mimusops elengi	46.24	45.77	-1.016	48.54	46.16	-4.90
Syzygium cumini	44.65	47.85	+7.167	47.13	45.53	-3.39



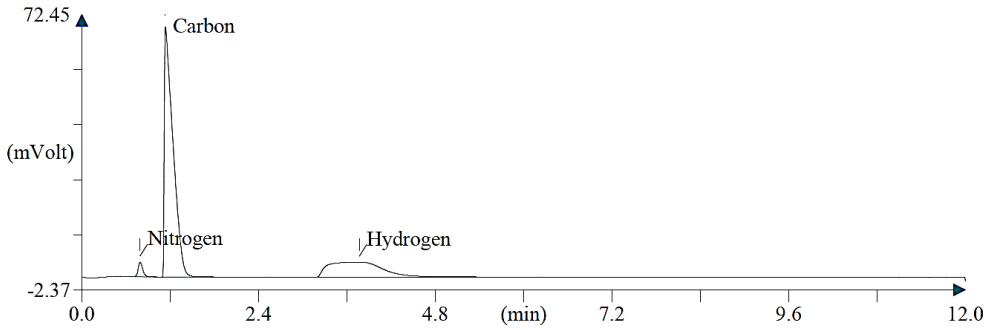
Element Name	%
Nitrogen	2.5000
Carbon	43.4254
Hydrogen	5.3701
Sulphur	0.

Figure 2.4.19a: Characteristic peaks obtained for *Terminalia arjuna* CD1 on CHNS analysis



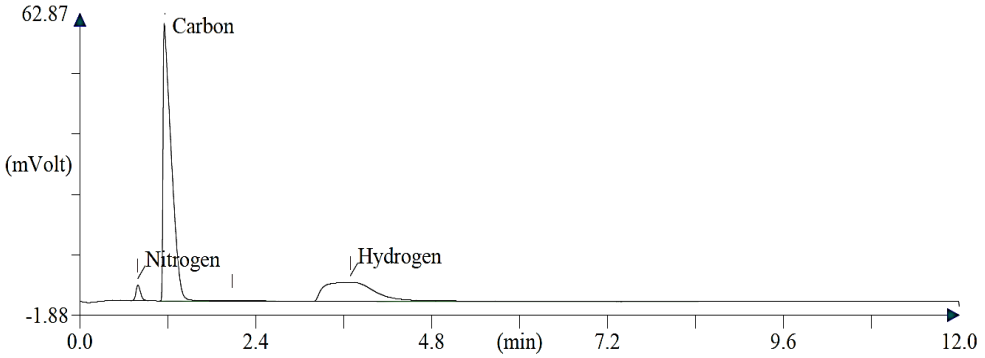
Element Name	%
Nitrogen	2.3856
Carbon	41.7033
Hydrogen	5.3712
Sulphur	0.

Figure 2.4.19b: Characteristic peaks obtained for *Terminalia arjuna* CD15 on CHNS analysis



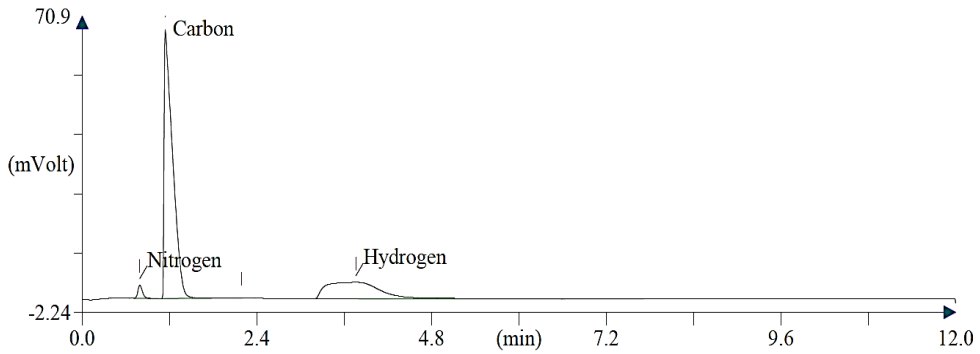
Element Name	%
Nitrogen	2.4330
Carbon	43.7405
Hydrogen	5.6416
Sulphur	0.

Figure 2.4.19c: Characteristic peaks obtained for *Terminalia arjuna* TD1 on CHNS analysis



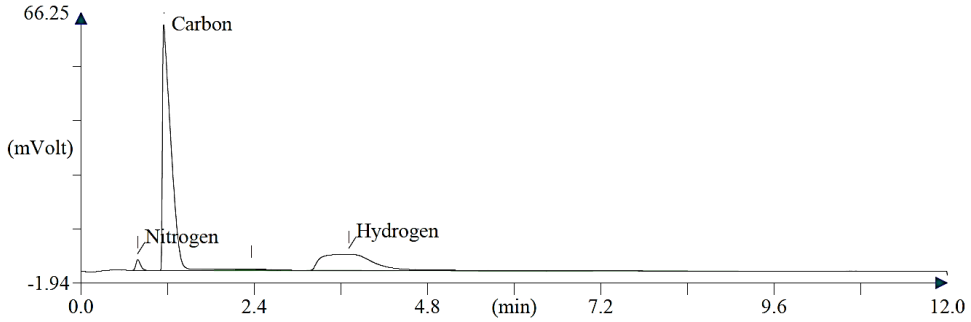
Element Name	%
Nitrogen	2.3468
Carbon	42.4269
Hydrogen	5.8641
Sulphur	0.

Figure 2.4.19d: Characteristic peaks obtained for *Terminalia arjuna* TD15 on CHNS analysis



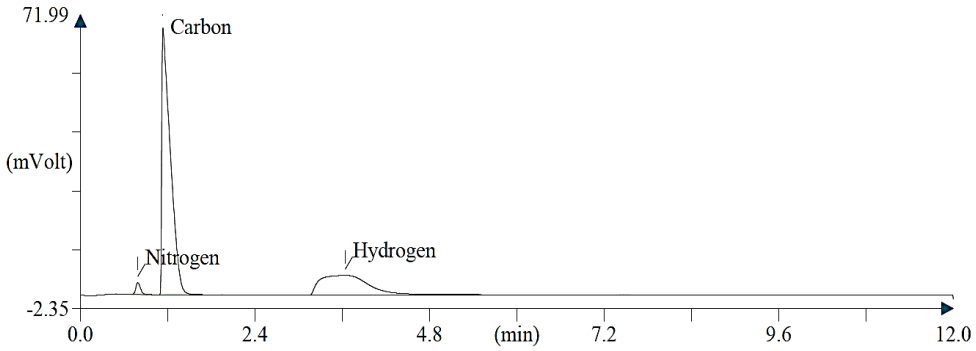
Element Name	%
Nitrogen	2.0408
Carbon	44.4354
Hydrogen	5.7061
Sulphur	0.

Figure 2.4.19e: Characteristic peaks obtained for *Swietenia macrophylla* CD1 on CHNS analysis



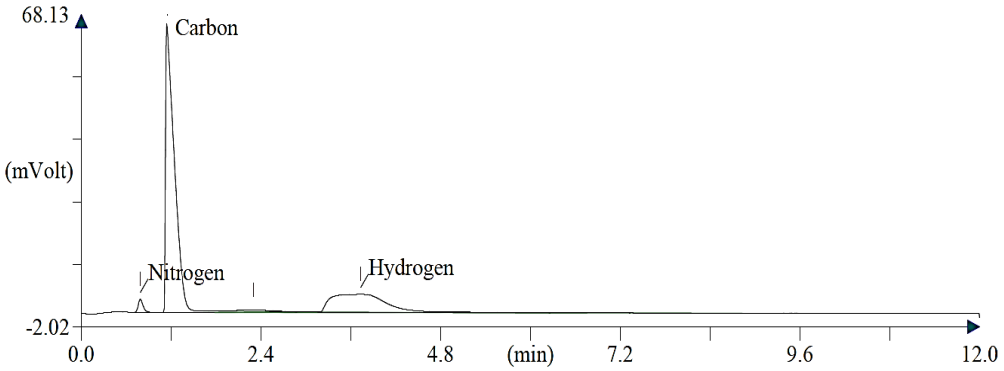
Element Name	%
Nitrogen	1.8913
Carbon	48.5988
Hydrogen	5.9915
Sulphur	0.

Fig 2.4.19f: Characteristic peaks obtained for *Swietenia macrophylla* CD15 on CHNS analysis



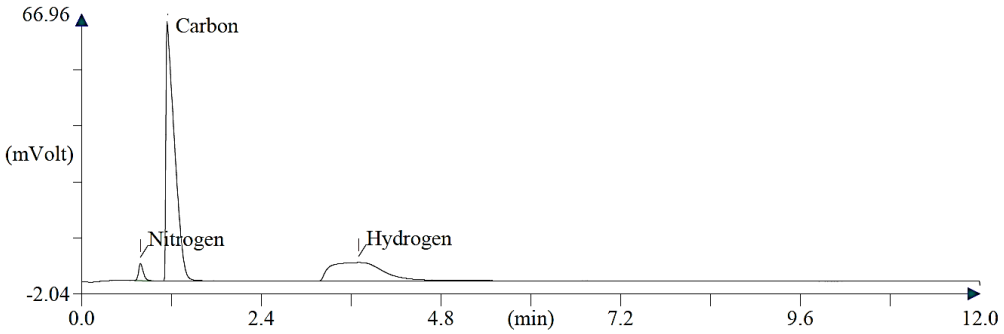
Element Name	%
Nitrogen	1.8172
Carbon	44.4724
Hydrogen	6.3814
Sulphur	0.

Figure 2.4.19g: Characteristic peaks obtained for *Swietenia macrophylla* TD1 on CHNS analysis



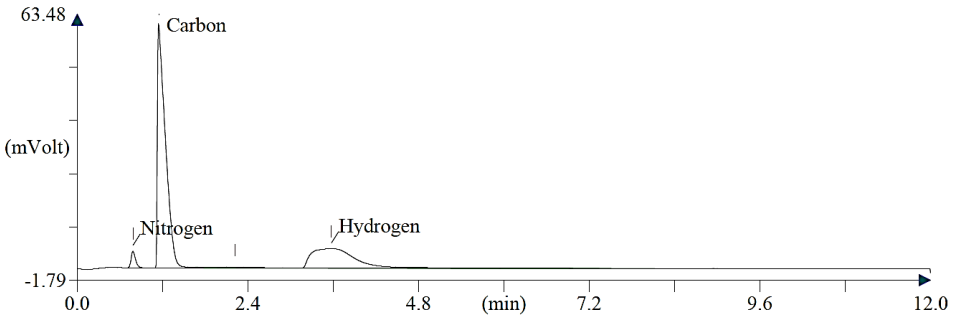
Element Name	%
Nitrogen	1.9869
Carbon	48.2534
Hydrogen	5.6640
Sulphur	0.

Figure 2.4.19h:. Characteristic peaks obtained for *Swietenia macrophylla* TD15 on CHNS analysis



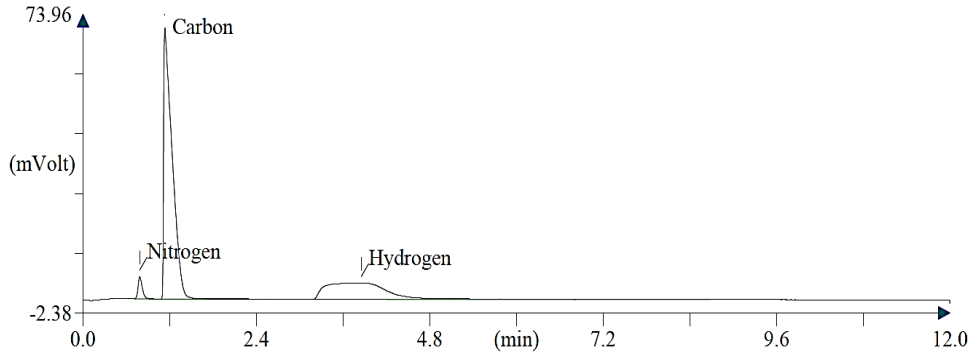
Element Name	%
Nitrogen	3.0312
Carbon	44.3796
Hydrogen	6.6006
Sulphur	0.

Figure 2.4.19i: Characteristic peaks obtained for *Pongamia pinnata* CD1 on CHNS analysis



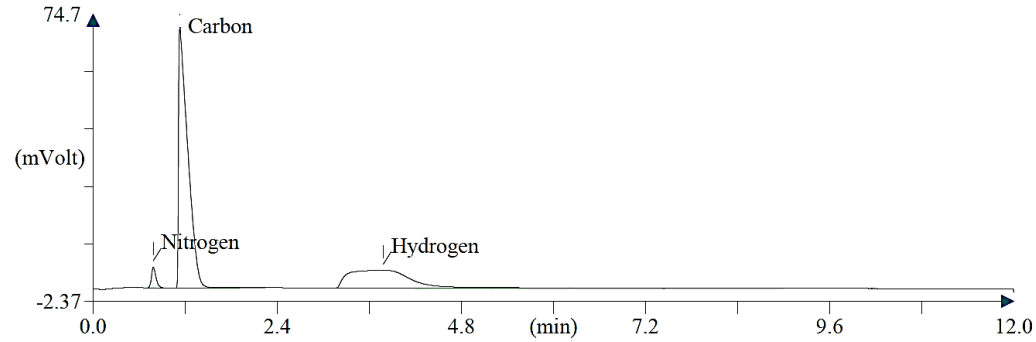
Element Name	%
Nitrogen	3.1852
Carbon	45.5496
Hydrogen	6.1209
Sulphur	0.

Figure 2.4.19j: Characteristic peaks obtained for *Pongamia pinnata* CD15 on CHNS analysis



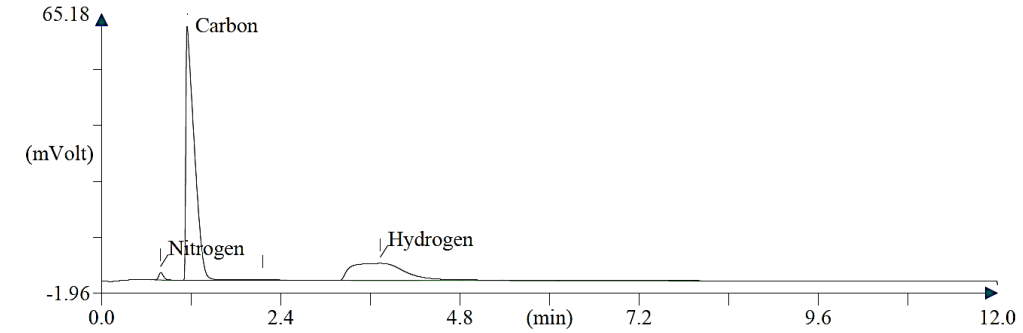
Element Name	%
Nitrogen	3.5868
Carbon	44.1559
Hydrogen	6.2482
Sulphur	0.

Figure 2.4.19k: Characteristic peaks obtained for *Pongamia pinnata* TD1 on CHNS analysis



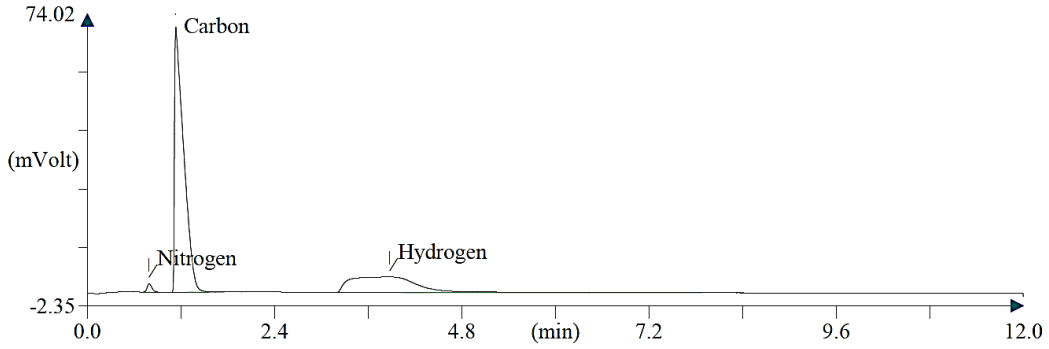
Element Name	%
Nitrogen	3.4638
Carbon	43.5049
Hydrogen	6.6599
Sulphur	0.

Figure 2.4.19l: Characteristic peaks obtained for *Pongamia pinnata* TD15 on CHNS analysis



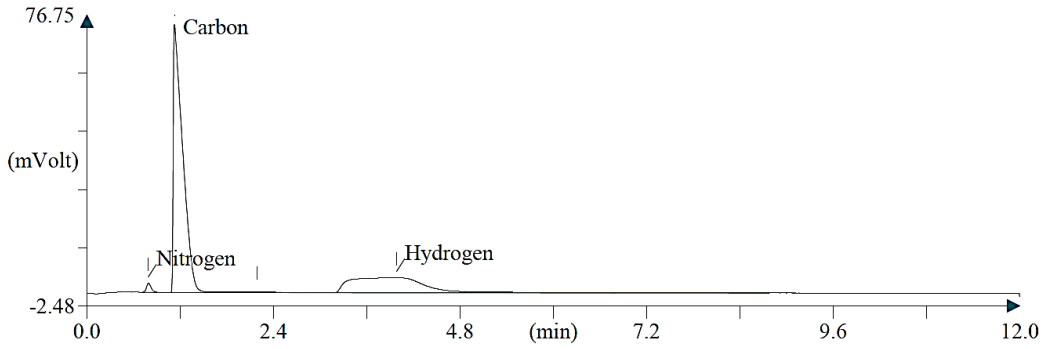
Element Name	%
Nitrogen	0.8502
Carbon	44.7602
Hydrogen	6.2449
Sulphur	0.

Figure 2.4.19m: Characteristic peaks obtained for *Simarouba glauca* CD1 on CHNS analysis



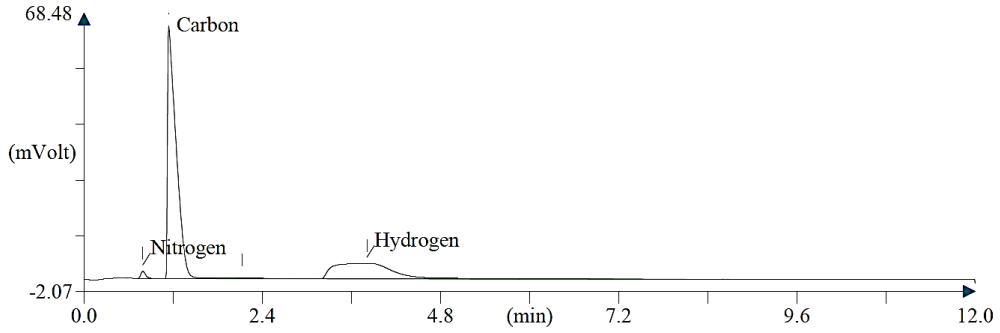
Element Name	%
Nitrogen	1.0581
Carbon	44.7616
Hydrogen	6.2589
Sulphur	0.

Figure 2.4.19n: Characteristic peaks obtained for *Simarouba glauca* CD15 on CHNS analysis



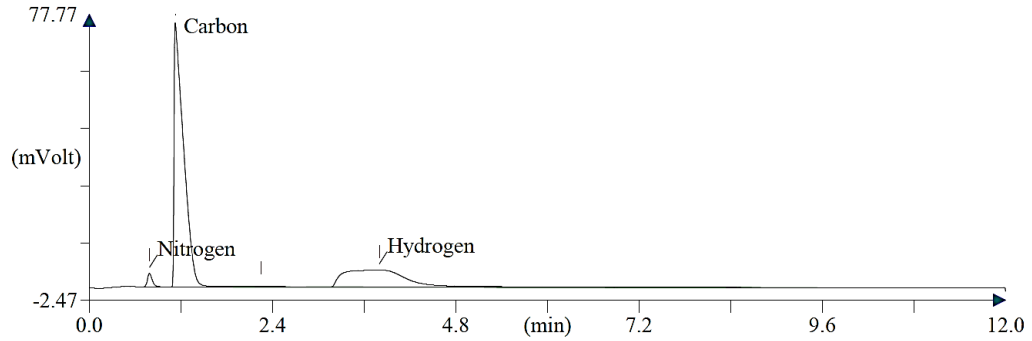
Element Name	%
Nitrogen	1.1136
Carbon	44.6579
Hydrogen	6.4162
Sulphur	0.

Figure 2.4.19o: Characteristic peaks obtained for *Simarouba glauca* TD1 on CHNS analysis



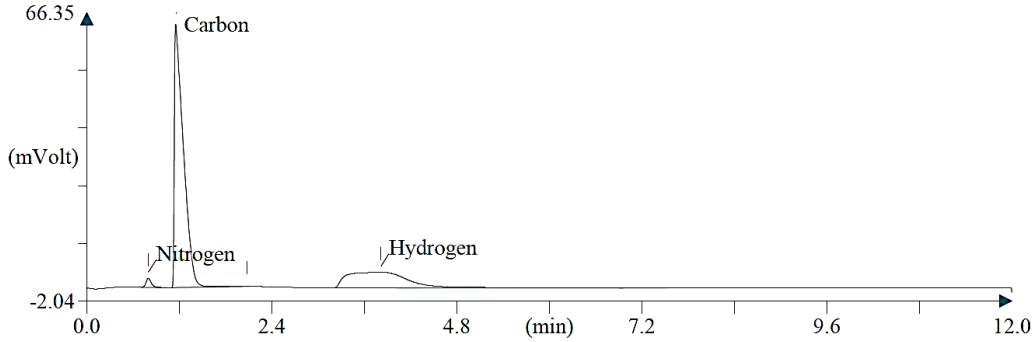
Element Name	%
Nitrogen	0.9033
Carbon	45.1963
Hydrogen	5.9996
Sulphur	0.

Figure 2.4.19p: Characteristic peaks obtained for *Simarouba glauca* TD15 on CHNS analysis



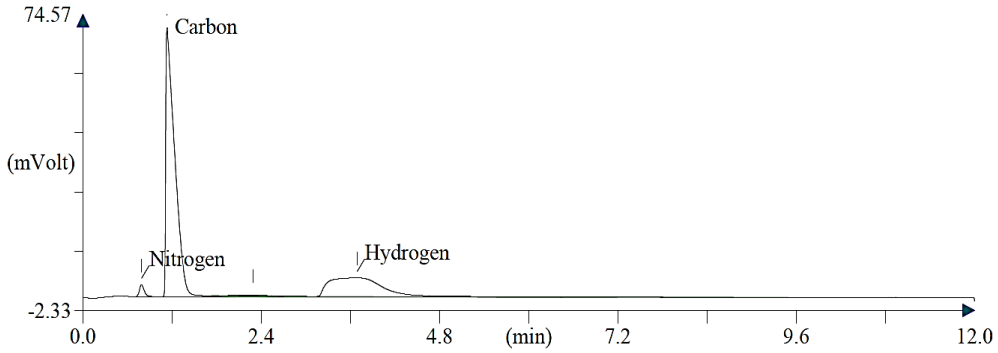
Element Name	%
Nitrogen	2.3133
Carbon	46.2455
Hydrogen	6.2227
Sulphur	0.

Figure 2.4.19q: Characteristic peaks obtained for *Mimusops elengi* CD1 on CHNS analysis



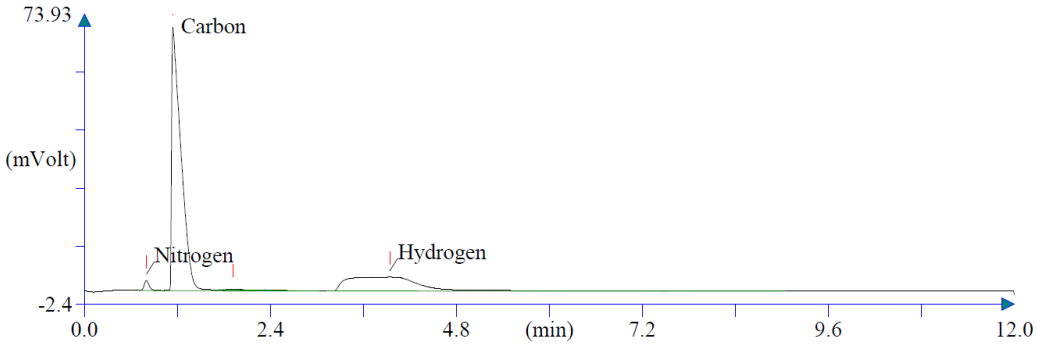
Element Name	%
Nitrogen	2.1419
Carbon	45.7716
Hydrogen	5.9329
Sulphur	0.

Figure 2.4.19r: Characteristic peaks obtained for *Mimusops elengi* CD15 on CHNS analysis



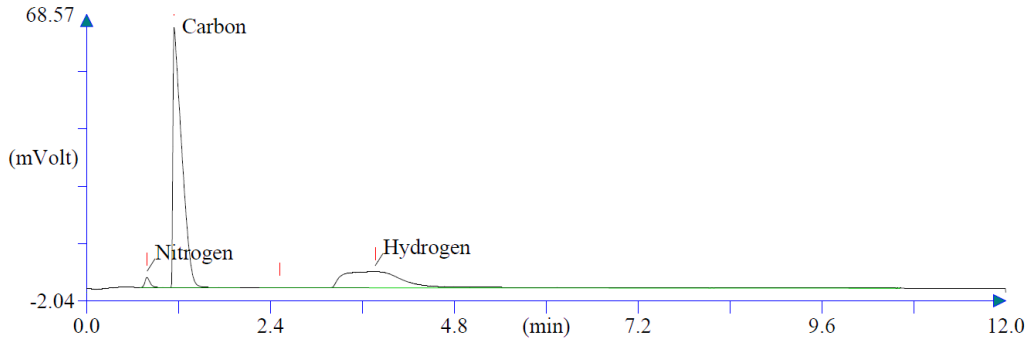
Element Name	%
Nitrogen	2.0510
Carbon	48.5424
Hydrogen	6.1573
Sulphur	0.

Figure 2.4.19s: Characteristic peaks obtained for *Mimusops elengi* TD1 on CHNS analysis



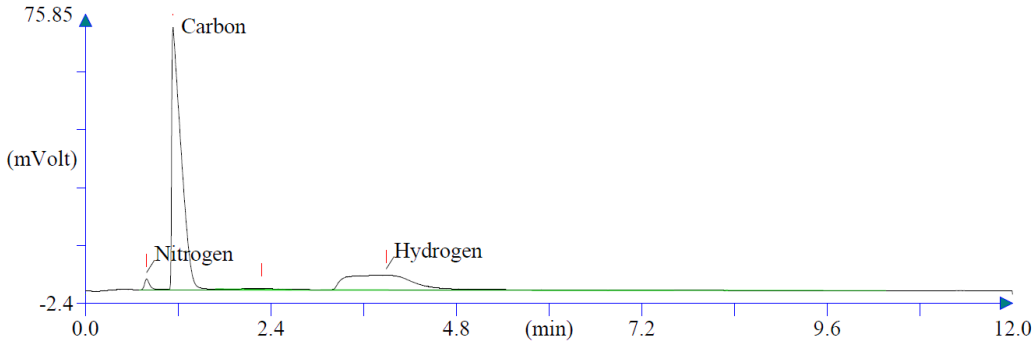
Element Name	%
Nitrogen	2.1695
Carbon	46.1601
Hydrogen	5.9530
Sulphur	0.

Figure 2.4.19t: Characteristic peaks obtained for *Mimusops elengi* TD15 on CHNS analysis



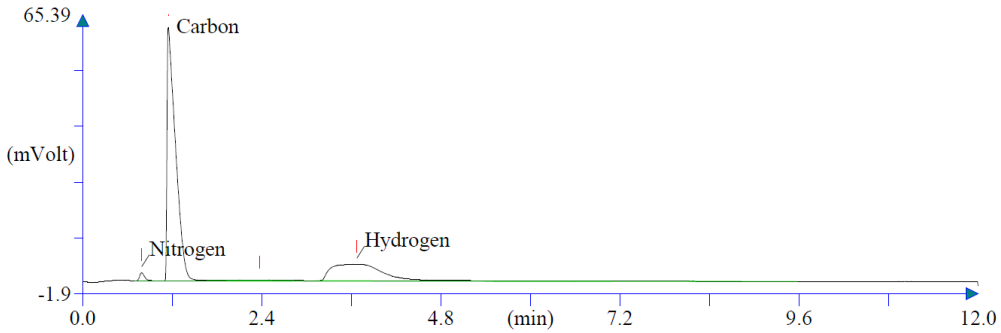
Element Name	%
Nitrogen	1.7009
Carbon	44.6542
Hydrogen	6.2614
Sulphur	0.

Figure 2.4.19u: Characteristic peaks obtained for *Syzygium cumini* CD1 on CHNS analysis



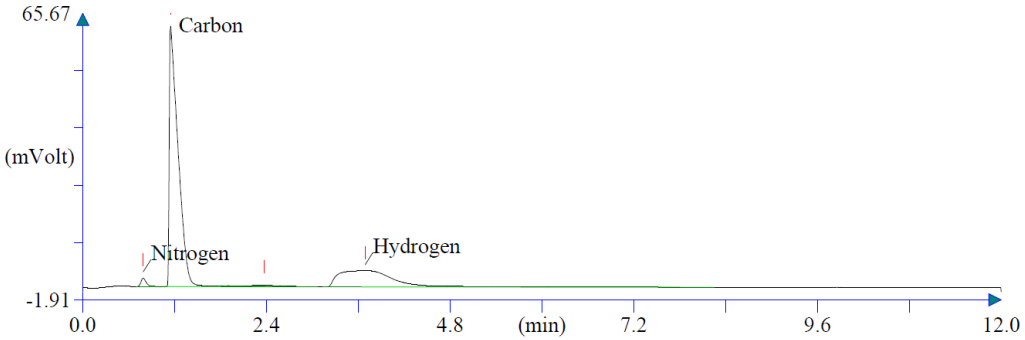
Element Name	%
Nitrogen	2.1233
Carbon	47.8567
Hydrogen	5.8715
Sulphur	0.

Figure 2.4.19v: Characteristic peaks obtained for *Syzygium cumini* CD15 on CHNS analysis



Element Name	%
Nitrogen	1.2687
Carbon	47.1363
Hydrogen	5.6274
Sulphur	0.

Figure 2.4.19w: Characteristic peaks obtained for *Syzygium cumini* TD1 on CHNS analysis



Element Name	%
Nitrogen	1.2490
Carbon	45.5329
Hydrogen	5.4993
Sulphur	0.

Figure 2.4.19x: Characteristic peaks obtained for *Syzygium cumini* TD15 on CHNS analysis

2.4.12 Plant micronutrients

There is a prominent change in the concentration of copper content under enhanced CO_2 conditions in all the plants. Percentage change in copper increased under

enriched CO_2 conditions in *T. arjuna*, *S. macrophylla*, *P. pinnata*, and *S. cumini* while a decrease in the concentration of copper is reported under enriched CO_2 conditions in *S. glauca* and *M. elengi*. The concentration of zinc increased under enhanced CO_2 levels compared to control in *T. arjuna*, *S. macrophylla*, *P. pinnata* while the percentage change in the concentration of zinc decreased under elevated CO_2 levels compared to control in *S. glauca*, *M. elengi*, *S. cumini*. **(Table 2.4.18 a and b).**

Table 2.4.18 a. Effects of elevated CO_2 on copper

Copper (%)		*Terminalia arjuna*	*Swietenia macrophylla*	*Pongamia pinnata*	*Simarouba glauca*	*Mimusops elengi*	*Syzygium cumini*
Control	**CD1**	46.7	53.6	50.15	29.9	34.1	47.3
	CD5	49.2	58.9	54.05	32.7	105.3	45.2
	CD10	37	22.8	29.9	26.2	30.8	36.4
	CD15	34.6	43.7	39.15	27.4	254.5	38.8
	% change	-25.91	-18.47	-14.34	-8.36	646.3	-17.97
Treatment	**TD1**	38.4	35.3	25	212	35.4	29.8
	TD5	39.2	45.1	42.8	31.2	42.6	31.2
	TD10	40.2	49.3	34.1	25.9	133	33.3
	TD15	42.4	44.3	45	28.2	32	35.9
	% change	10.41	25.49	80	-86.6	-9.6	20.46

[CD1- 1st day of control, CD5- 5th day of control, CD10- 10th day of control, CD15- 15th day of control, TD1- 1st day of treatment, TD5- 5th day of treatment, TD10- 10th day of treatment, TD15- 15th day of treatment]

Table 2.4.18 b. Effects of elevated CO_2 on zinc

Zinc (%)		*Terminalia arjuna*	*Swietenia macrophylla*	*Pongamia pinnata*	*Simarouba glauca*	*Mimusops elengi*	*Syzygium cumini*
Control	**CD1**	58.3	145	154.3	228.8	134.4	280.2
	CD5	64.3	54.3	133.1	145.4	149.7	187
	CD10	65.2	118	108.2	146.6	114.7	134.9
	CD15	60.4	92.8	155.9	155.9	287.8	179.1
	% change	3.6	-36	1.03	-31.86	114.13	-36.08
Treatment	**TD1**	45.3	87.6	35.5	312.3	123.2	184.8
	TD5	47.2	148.4	214.4	224.2	181.7	135.3
	TD10	55.3	169.2	191.5	92	166.7	122.6
	TD15	57.2	118.6	187	85.8	172.9	147.1
	% change	26.26	35.38	426.7	-72.52	40.34	-20.4

[CD1- 1st day of control, CD5- 5th day of control, CD10- 10th day of control, CD15- 15th day of control, TD1- 1st day of treatment, TD5- 5th day of treatment, TD10- 10th day of treatment, TD15- 15th day of treatment]

2.4.13 FTIR analysis

The study revealed that in *T. arjuna*, an FTIR peak at 1647.61 cm^{-1} is present in the CO_2-treated condition (absent in the control) which can be due to the presence of alkenyl C=C stretch. The presence of O=C=O stretching at FTIR peak 2360.10 cm^{-1} confirms CO_2. The presence of nitrate ions is indicated by an FTIR peak at 1372.41 cm^{-1} in the CO_2-treated condition which is absent in the control. FTIR peak at 894.19 cm^{-1} on the 15^{th} day of CO_2 treatment represents COC and CCH stretching. The broadening structure of the peak represents a disordered structure **(Figure 2.4.20 a, b.)**

In *S. macrophylla,* an FTIR peak at 1732.67 cm^{-1} in the control on the final day of CO_2 treatment is present indicating the presence of carbonyl compounds such as ketones and aldehydes, and is absent in the treatment. A prominent peak at 2360 cm^{-1} indicates the presence of Carbon dioxide. A very distinct peak at 1069.12 cm^{-1} represents S=O stretching, and C=O stretching indicates the presence of sulfoxide and a primary alcohol. Different peaks in the range of 895-885 cm^{-1} are attributed to the presence of an alkene group indicating C=C bending. The peaks at 2923 cm^{-1} are attributed to NH stretching vibrations of amine salt **(Figure 2.4.20 c, d.)**

In *P. pinnata* an FTIR peak at 3411.54 cm^{-1} in the CO_2 treatment after 15 days is due to the presence of OH stretching indicating an alcohol group. The prominent peak at 2360 cm^{-1} is attributed to the presence of Carbon dioxide. Absorbance at wave numbers of 1541.80 cm^{-1} could be assigned to the presence of vibrations of the nitrogen-hydrogen single bond. FTIR peak at a wavenumber of 1030.87 cm^{-1} was contributed to stretching vibrations of S=O, indicating sulfoxide group **(Figure 2.4.20 e, f)**

In *S. glauca*, after 15 days of CO_2 treatment, an FTIR peak at 3359.48 cm^{-1} contributes to OH stretching indicating the presence of a strong alcohol bond. CH stretching and the presence of an alkane group are relevant from the FTIR peak at 2919.54 $cm^{-1.}$ The presence of Carbon dioxide is confirmed by O=C=O stretching observed from the FTIR peak at 2359.78 $cm^{-1.}$ The presence of a halo compound is also observed**. (Figure 2.4.20 g, h)**

In *M. elengi* an FTIR peak at 3417.9 cm^{-1} contributes to OH stretching indicating the presence of an alcohol group. A very distinct peak at 2922.26 cm^{-1} contributes to CH

stretching showing an alkane group. The prominent peak at 2359.67 cm^{-1} is attributed to O=C=O stretching showing the presence of Carbon dioxide. Peaks in the range of 1650-1566 cm^{-1} indicate the presence of a cyclic alkene which is attributed to C=C stretching. Peaks in the range of 690-515 cm^{-1} confirm the presence of a halo compound **(Figure 2.4.20 i, j)**

In *S. cumini*, after 15 days of CO_2 treatment, the FTIR peak at 3428.10 cm^{-1} represents OH stretching indicating the alcohol group. The FTIR peak in the range of 3000-2840 cm^{-1} contributes to CH stretching indicating the alkane group. The prominent peak at 2361.15 cm^{-1} is attributed to O=C=O stretching indicating the presence of Carbon dioxide. The relevant peak in the range of 1150-1085 cm^{-1} represents C=O stretching indicating the aliphatic ether group. FTIR peak at 535.11 cm^{-1} represents a halo compound **(Figure 2.4.20 k,l)**

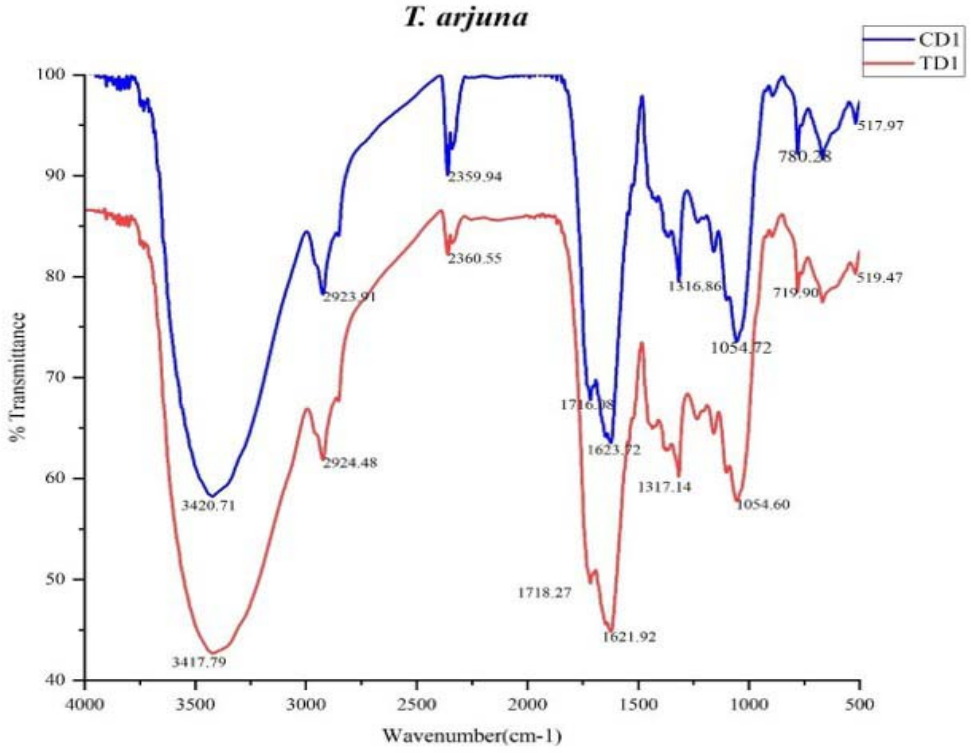


Figure 2.4.20a: Characteristic peaks obtained for *T. arjuna* CD1 and TD1 on FTIR analysis

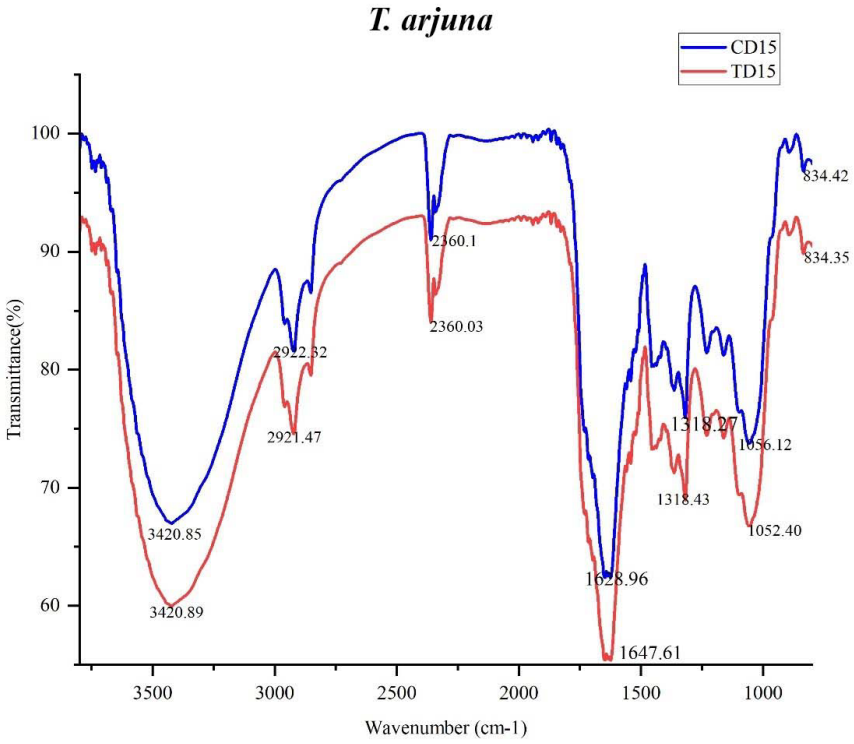


Figure 2.4.20b: Characteristic peaks obtained for *T. arjuna* CD15 and TD15 on FTIR analysis

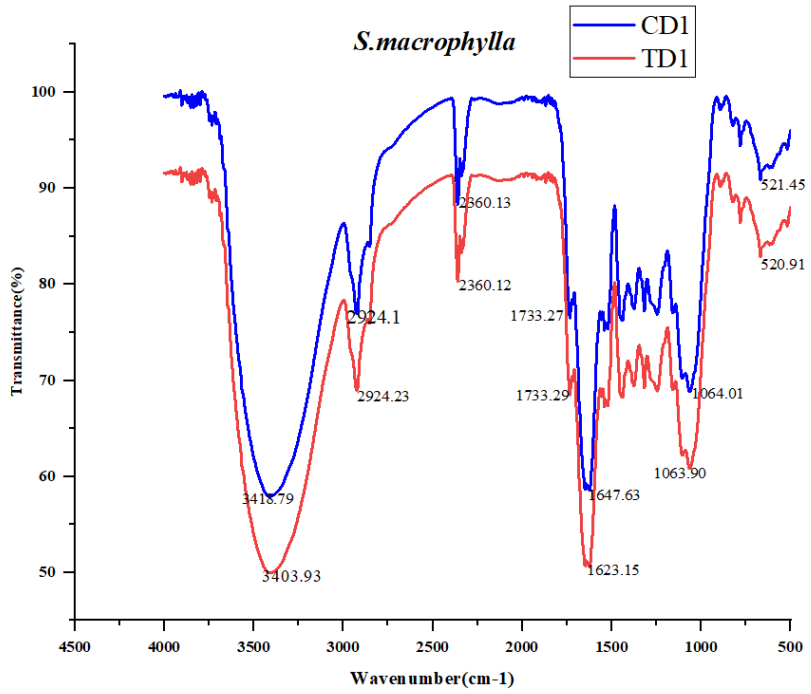


Figure 2.4.20c: Characteristic peaks obtained for *S. macrophylla* CD1 and TD1 on FTIR analysis.

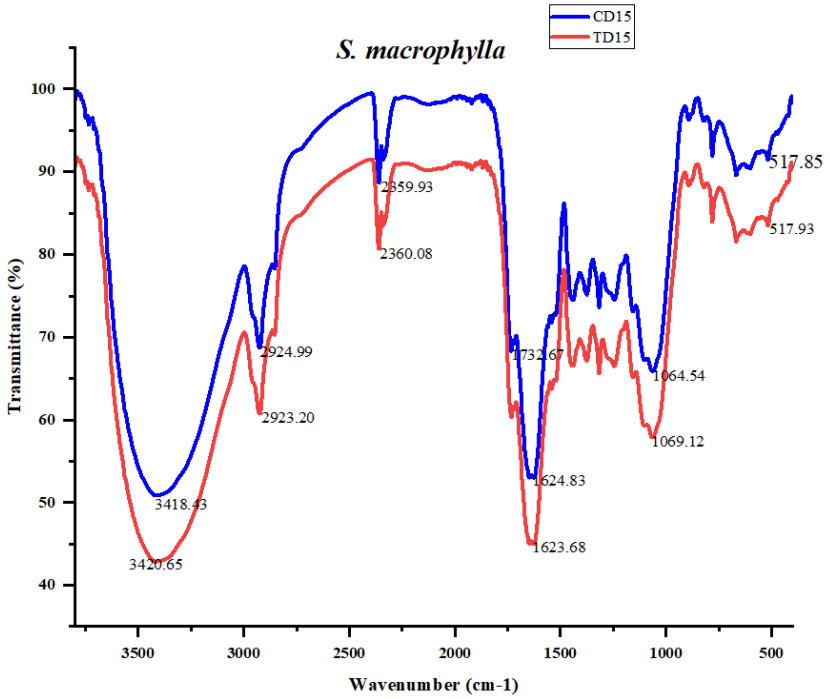


Figure 2.4.20d: Characteristic peaks obtained for *S. macrophylla* CD15 and TD15 on FTIR analysis

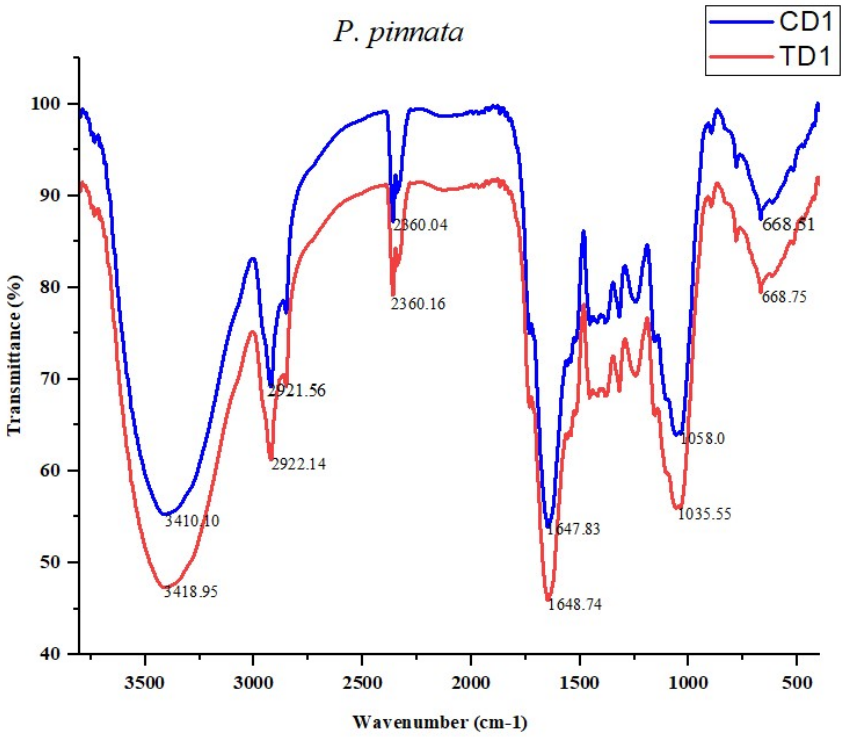


Figure 2.4.20e: Characteristic peaks obtained for *P. pinnata* CD1 and TD1 on FTIR analysis

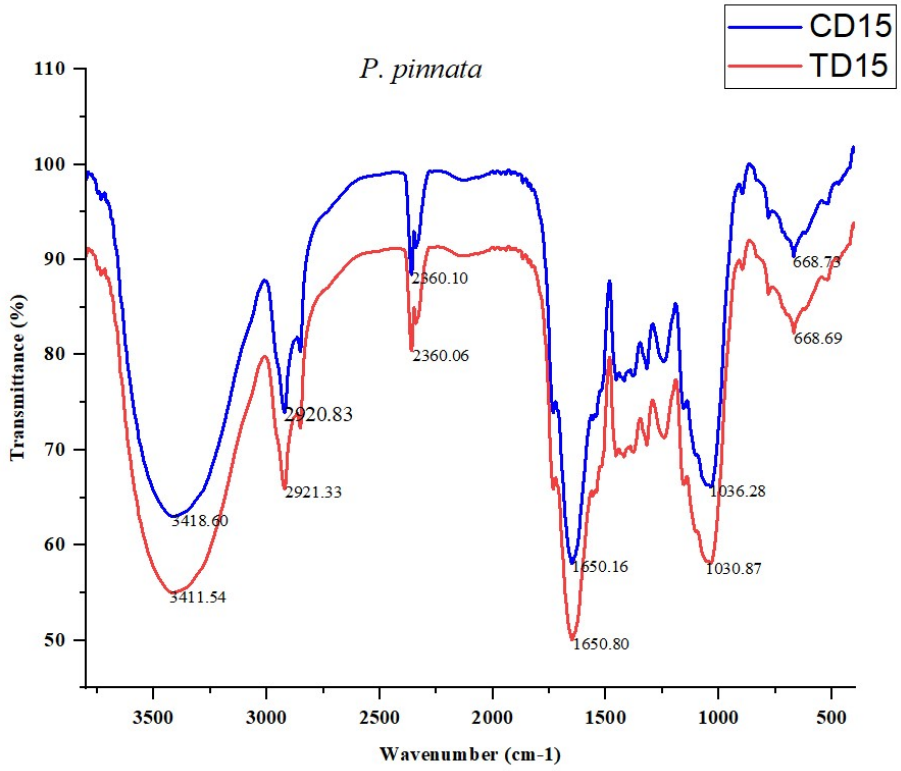


Figure 2.4.20f: Characteristic peaks obtained for *P. pinnata* CD15 and TD15 on FTIR analysis

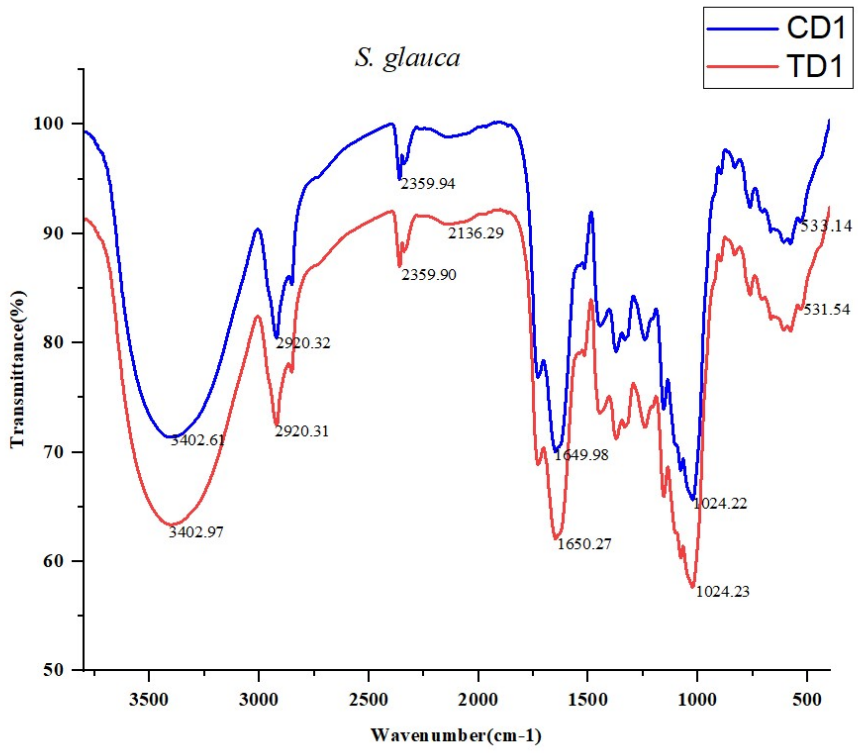


Figure 2.4.20g: Characteristic peaks obtained for *S. glauca* CD1 and TD1 on FTIR analysis

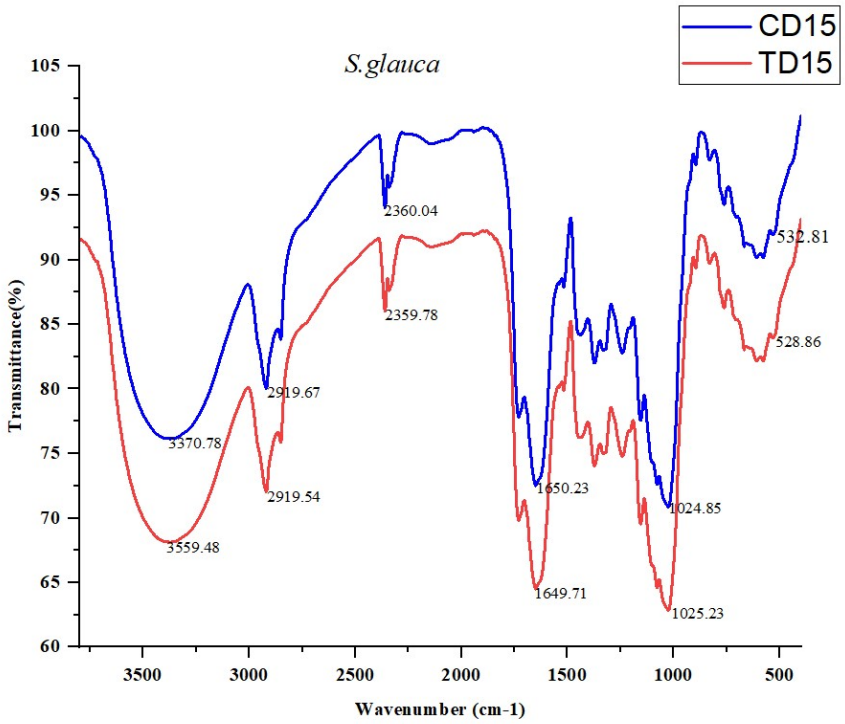


Figure 2.4.20h: Characteristic peaks obtained for *S. glauca* CD15 and TD15 on FTIR analysis

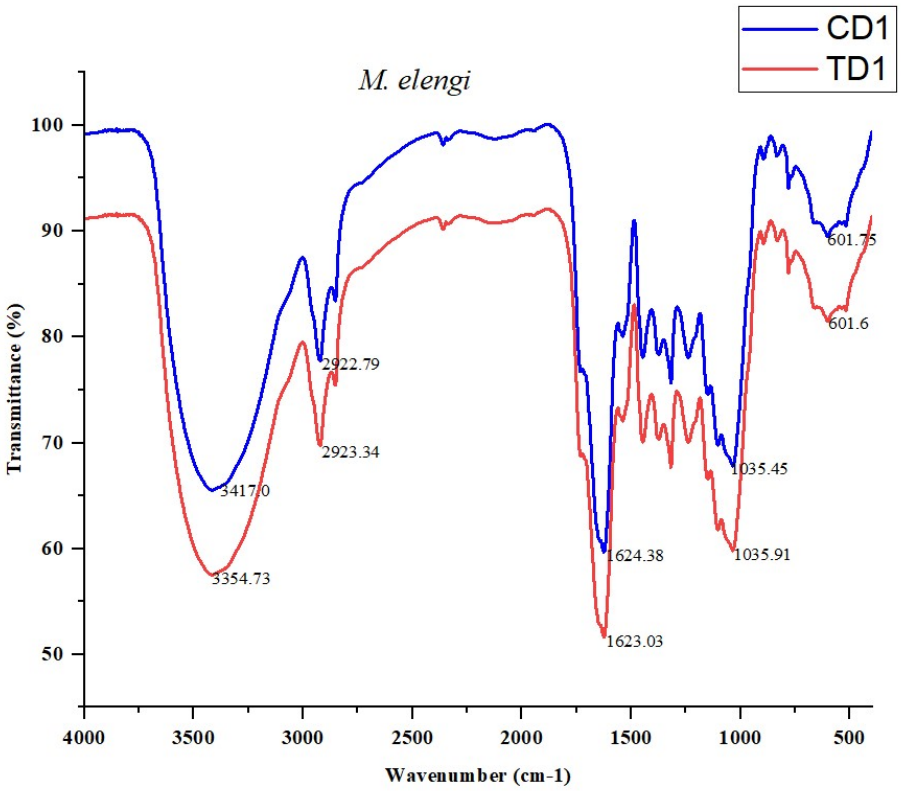


Figure 2.4.20i: Characteristic peaks obtained for *M. elengi* CD1 and TD1 on FTIR analysis

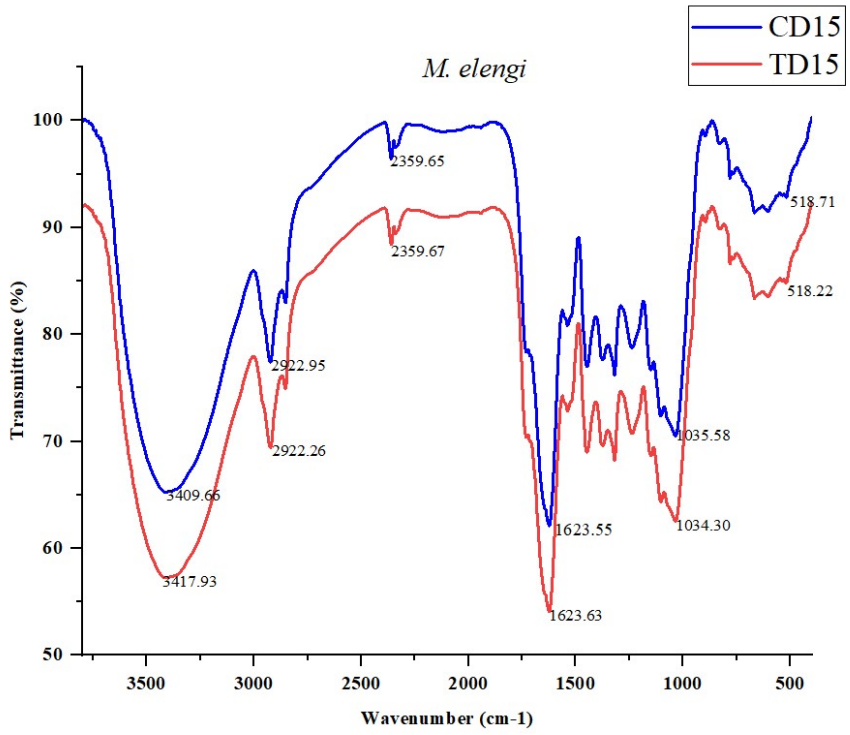


Figure 2.4.20j: Characteristic peaks obtained for *M. elengi* CD15 and TD15 on FTIR analysis

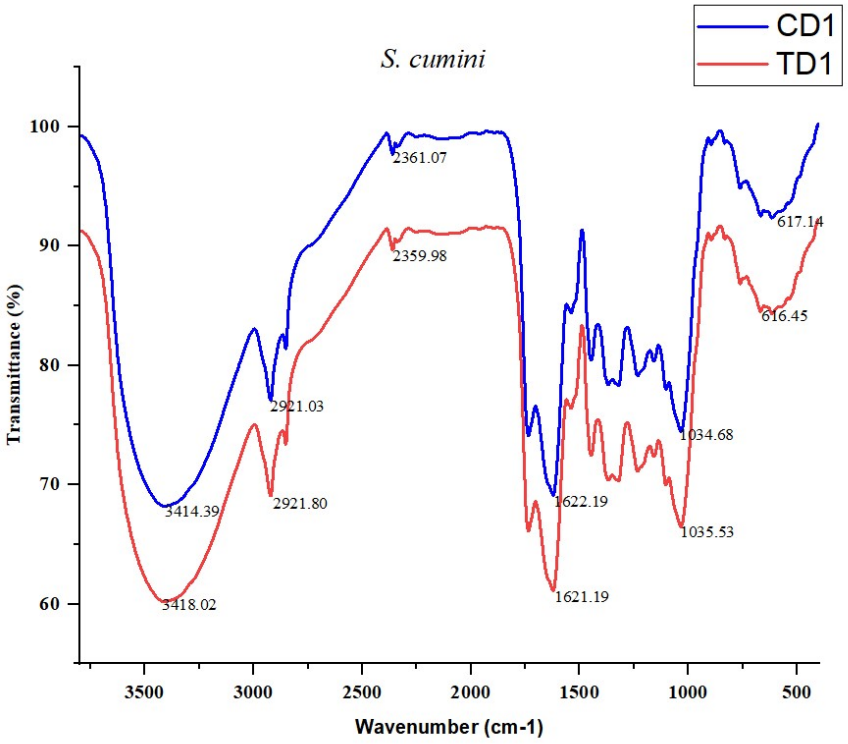


Figure 2.4.20k: Characteristic peaks obtained for *S. cumini* CD1 and TD1 on FTIR analysis

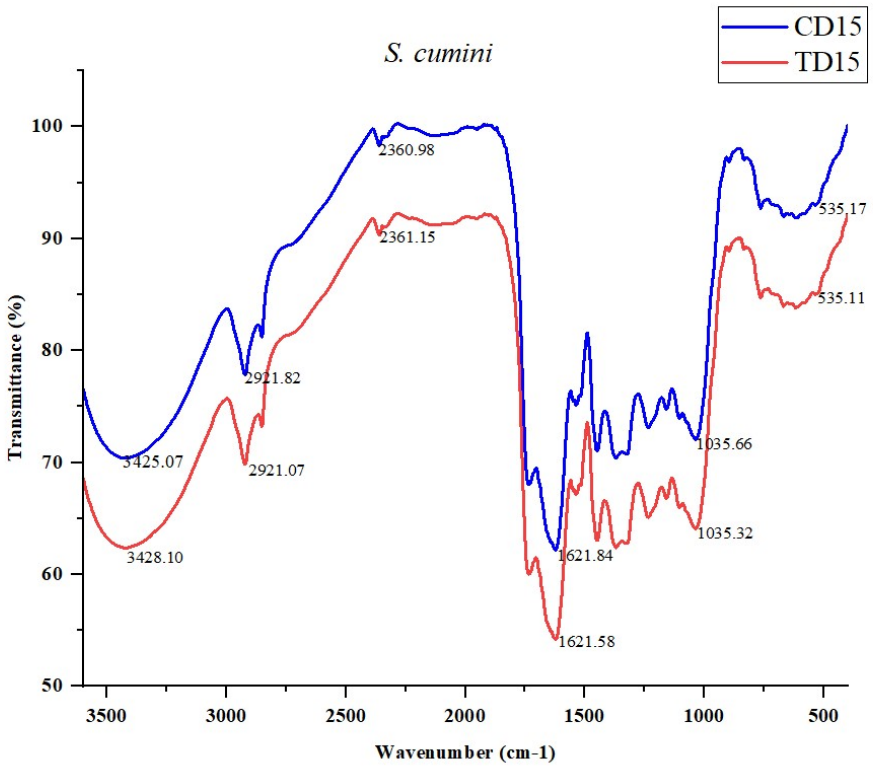


Figure 2.4.20l: Characteristic peaks obtained for *S. cumini* CD15 and TD15 on FTIR analysis

2.4.14 Soil characteristics

The soil moisture increased in TC compared to CC in *P. pinnata*, *M. elengi,* and *S. cumini* whereas soil moisture decreased under enhanced levels of CO_2 compared to control in *T. arjuna*, *S. macrophylla* and *S. glauca* **(Table 2.4.19 and Figure 2.4.21).** The decrease in soil moisture in *T. arjuna* and *S. macrophylla* is statistically significant (w=1, p = 0.05) **(Annexure 12).**

Soil pH increased from 5.638 on the 1st day to 5.883 on the final day in TC having *S. macrophylla*. Similarly, soil pH increased from 5.323 on the first day to 6.595 on the final day in TC having *P. pinata*. The decrease in soil pH in the TC compared to CC in *T. arjuna*, *S. glauca M. elengi,* and *S. cumini* indicates that the soil has turned acidic from basic **(Table 2.4.20 and Figure 2.4.22).** The change in soil pH is statistically significant in *S. glauca* (w=16, p < 0.028) **(Annexure 12).**

Soil carbon increased by 50.89% in TC compared to CC having *S. cumini* while it decreased in all other sets under enriched CO_2 conditions compared to the control. **(Table 2.4.21 and Figure 2.4.23).** The changes in soil carbon were not statistically significant in the treatments **(Annexure 12).**

Soil nitrogen increased in *S. macrophylla and P. pinata* by 35.71% and 6.45% respectively under elevated CO_2 conditions compared to CC while a reduction in the concentration of nitrogen has been observed in all other plants. Similarly, phosphorous content in soil increased by 46.31% and 10.36% in TC having *P. pinnata* and *S. cumini*, compared to CC. In contradiction to this result, a reduction in soil phosphorous by 8.94%, 1.57%, 27.12%, and 22.84% has been observed in sets having *T. arjuna*, *S. macrophylla*, *S. glauca*, and *M. elengi* respectively compared to the initial day.

Reduction in potassium is more prominent in control conditions compared to treatment. A 33.66% reduction in soil potassium is observed in *S. cumini* in elevated CO_2 condition followed by 30.11% in *P. pinnata,* 23.52% in *M. elengi*, 16.36% in *S. macrophylla*, 6.16% in *T. arjuna* and 3.47% in *S. glauca* **(Table 2.4.22).**

Table 2.4.19: Variations in soil moisture (%) having plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Terminalia arjuna					
C1	23.267	20.644	**T1**	24.569	24.075
C2	24.186	19.673	**T2**	27.358	23.662
C3	16.251	20.660	**T3**	23.113	21.019
C4	15.596	22.444	**T4**	22.330	25.734
Avg	19.825	20.855	**Avg**	24.343	23.623
SD	4.528	1.155	**SD**	2.214	1.953
% Change		+5.195	**% Change**		-2.957
Swietenia macrophylla					
C1	14.257	22.827	**T1**	19.436	23.366
C2	14.864	20.740	**T2**	16.960	26.498
C3	12.905	20.070	**T3**	14.872	25.977
C4	15.536	23.687	**T4**	19.270	25.939
Avg	14.390	21.831	**Avg**	17.634	25.445
SD	1.120	1.705	**SD**	2.161	1.409
% Change		+51.70	**% Change**		+44.29
Pongamia pinnata					
C1	18.806	19.866	**T1**	21.659	23.987
C2	23.012	20.356	**T2**	21.078	20.178
C3	18.532	21.731	**T3**	17.581	23.039
C4	25.195	25.307	**T4**	17.872	20.351
Avg	21.386	21.815	**Avg**	19.548	21.889
SD	3.264	2.458	**SD**	2.120	1.916
% Change		-24.69	**% Change**		+11.97

Simarouba glauca					
C1	9.100	13.569	**T1**	15.498	16.717
C2	17.383	18.750	**T2**	12.133	17.503
C3	14.629	18.585	**T3**	9.316	16.849
C4	16.087	19.630	**T4**	8.384	17.510
Avg	14.300	17.634	**Avg**	11.333	17.145
SD	3.644	2.748	**SD**	3.202	0.421
% Change		+623.31	**% Change**		+51.28
Mimusops elengi					
C1	3.686	5.010	**T1**	3.704	6.383
C2	3.760	4.535	**T2**	4.019	5.302
C3	3.982	4.959	**T3**	4.813	6.822
Avg	3.809	3.626	**Avg**	4.178	6.169
SD	0.154	0.261	**SD**	0.572	0.782
% Change		-4.804	**% Change**		+47.65
Syzygium cumini					
C1	12.463	16.845	**T1**	14.113	25.429
C2	13.262	14.855	**T2**	18.242	22.089
C3	17.531	14.641	**T3**	16.360	16.303
Avg	14.419	15.447	**Avg**	16.238	21.274
SD	2.725	1.215	**SD**	2.067	4.617
% Change		+7.129	**% Change**		+31.01

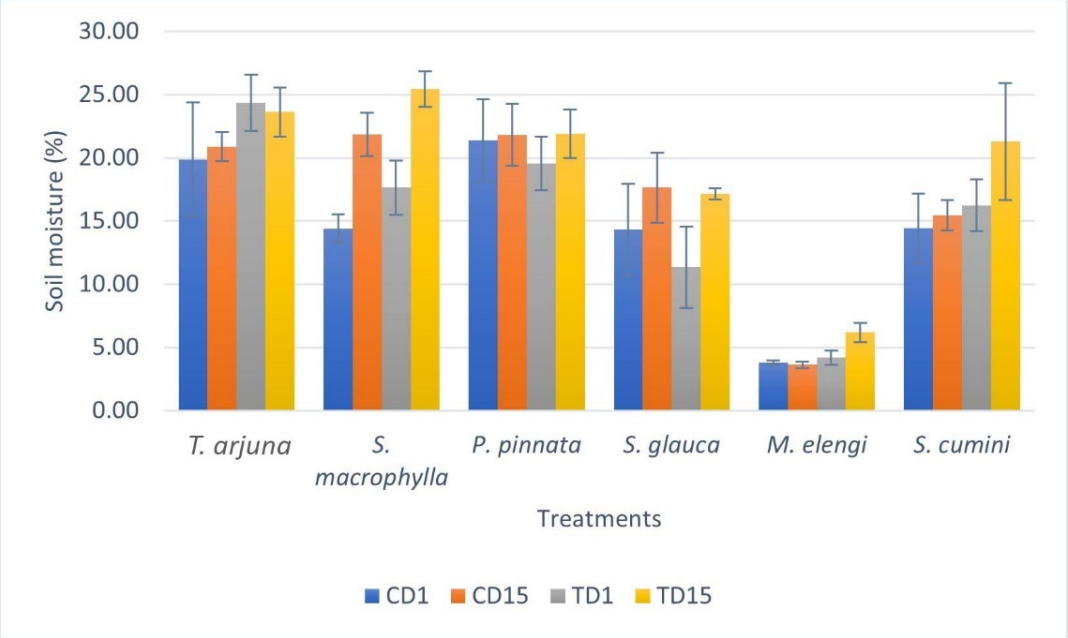


Figure 2.4.21: Effects of elevated CO_2 on soil moisture (%)

Table 2.4.20: Variations in soil pH having plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Terminalia arjuna					
C1	6.34	5.5	**T1**	6.56	5.73
C2	6.34	5.93	**T2**	6.83	5.1
C3	6.84	5.4	**T3**	6.62	5.23
C4	6.05	5.83	**T4**	5.63	4.92
Avg	6.393	5.665	**Avg**	6.41	5.245
SD	0.328	0.255	**SD**	0.533	0.347
% Change		-11.37	**% Change**		-18.25
Swietenia macrophylla					
C1	5.59	6.36	**T1**	5.7	5.91
C2	7.26	5.06	**T2**	6.02	6.13
C3	5.8	5.63	**T3**	5.07	5.64
C4	6.09	5.24	**T4**	5.76	5.85
Avg	6.185	5.57	**Avg**	5.638	5.883
SD	0.745	0.576	**SD**	0.403	0.202
% Change		-9.943	**% Change**		+4.345
Pongamia pinnata					
C1	6.47	6.54	**T1**	5.76	5.68
C2	6.59	8.55	**T2**	6.37	6.36
C3	6.7	7.63	**T3**	5.83	6.43
C4	6.58	7.84	**T4**	3.33	7.91
Avg	6.585	7.64	**Avg**	5.323	6.595
SD	0.094	0.832	**SD**	1.356	0.94
% Change		+16.02	**% Change**		+24.06
Simarouba glauca					
C1	7.62	6.49	**T1**	7.1	6.12
C2	11.08	6.92	**T2**	6.57	6
C3	9.88	6.4	**T3**	6.54	6.03
C4	10.32	6.25	**T4**	6.62	6.16
Avg	9.725	6.515	**Avg**	6.708	6.078
SD	1.488	0.288	**SD**	0.264	0.075
% Change		-33.00	**% Change**		-9.391
Mimusops elengi					
C1	5.56	5.76	**T1**	6.1	6.33
C2	5.57	6.14	**T2**	6.32	6.36
C3	5.38	6.3	**T3**	4.77	5.67
Avg	5.503	6.067	**Avg**	5.73	6.12
SD	0.107	0.28	**SD**	0.83863	0.39
% Change		+10.23	**% Change**		+6.806
Control	D1	D15	**Treatment**	D1	D15

Syzygium cumini					
C1	6.13	6.4	**T1**	6.46	6.23
C2	6.69	5.98	**T2**	6.92	6.12
C3	6.14	6.98	**T3**	6.35	6.48
Avg	6.32	6.453	**Avg**	6.58	6.28
SD	0.320	0.502	**SD**	0.30	0.18
% Change		+2.104	**% Change**		-4.559

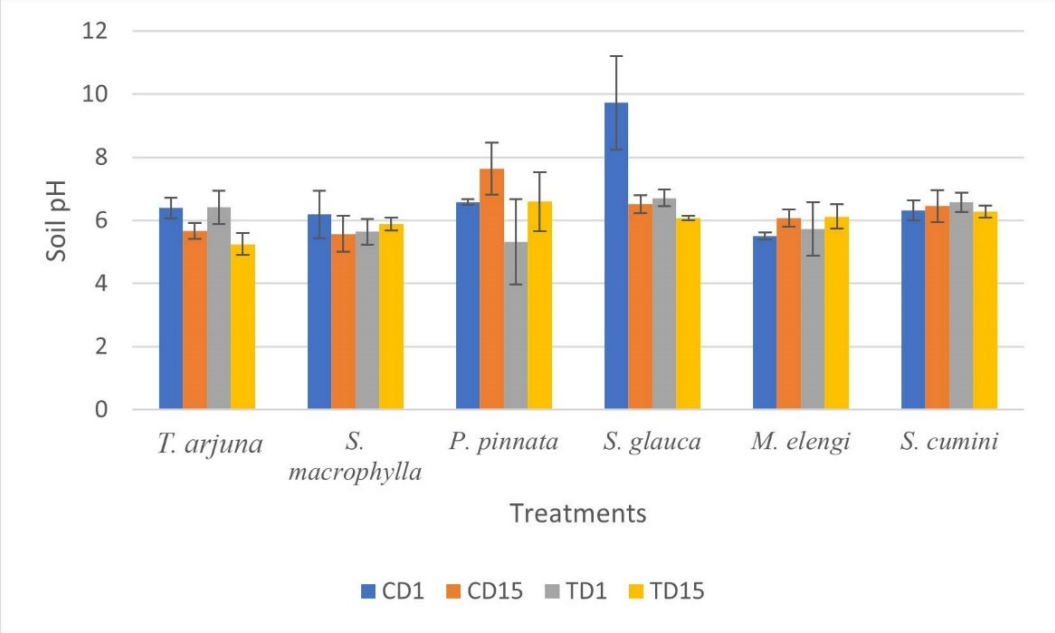


Figure 2.4.22: Effect of elevated CO_2 on soil pH

Table 2.4.21: Variations in soil carbon (%) having plants under elevated levels of CO_2

Control	D1	D15	Treatment	D1	D15
Terminalia arjuna					
C1	2.36	3.264	**T1**	5.51	4.896
C2	3.168	3.672	**T2**	5.51	5.712
C3	4.344	2.856	**T3**	1.968	6.528
C4	4.344	5.712	**T4**	1.968	2.856
Avg	3.554	3.876	**Avg**	3.739	4.998
SD	0.970	1.269	**SD**	2.045	1.576
% Change		+9.060	**% Change**		+33.67
Swietenia macrophylla					
C1	2.808	6.216	**T1**	6	4.272
C2	4.416	5.808	**T2**	6.816	7.368
C3	4.008	3.12	**T3**	5.208	6.216

C4	3.6	4.272	**T4**	8.808	4.656
Avg	3.708	4.854	**Avg**	6.708	5.628
SD	0.686	1.427	**SD**	1.546	1.433
% Change		+30.90	**% Change**		-16.10
Pongamia pinnata					
C1	1.44	8.4	**T1**	6.48	7.68
C2	2.4	5.49	**T2**	4.32	5.28
C3	1.92	8.64	**T3**	4.6	6.24
C4	4.63	3.6	**T4**	3.36	8.4
Avg	2.598	6.533	**Avg**	4.690	6.900
SD	1.411	2.423	**SD**	1.306	1.405
% Change		+151.5	**% Change**		+47.12
Simarouba glauca					
C1	5.13	5.189	**T1**	2.908	4.571
C2	4.03	8.48	**T2**	5.27	10.03
C3	2.12	9.49	**T3**	4.77	8.44
C4	5.23	9.69	**T4**	4.61	7.92
Avg	4.128	8.212	**Avg**	4.390	7.740
SD	1.445	2.084	**SD**	1.027	2.296
% Change		+98.93	**% Change**		+76.30
Mimusops elengi					
C1	6.302	2.195	**T1**	3.55	2.173
C2	5.66	5.687	**T2**	4.8	6.056
C3	7.815	1.246	**T3**	7.53	5.071
Avg	6.592	3.043	**Avg**	5.293	4.433
SD	1.106	2.339	**SD**	2.035	2.019
% Change		-53.83	**% Change**		-16.24
Syzygium cumini					
C1	2.575	3.21	**T1**	3.84	3.33
C2	4.172	1.73	**T2**	6.857	2.15
C3	0.816	2.62	**T3**	5.347	2.41
Avg	2.521	2.520	**Avg**	5.348	2.630
SD	1.679	0.745	**SD**	1.509	0.620
% Change		-0.039	**% Change**		+50.89

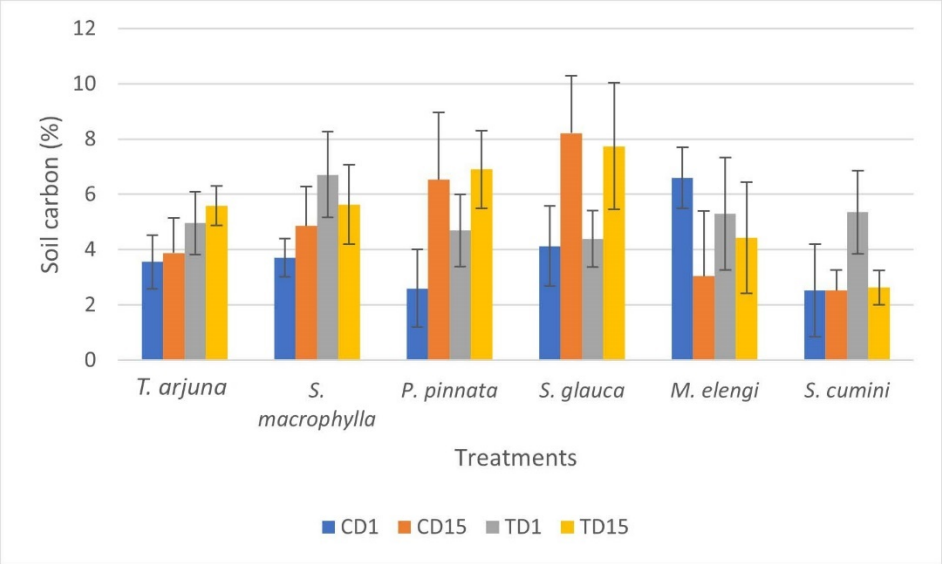


Figure: 2.4.23: Effects of elevated CO_2 on soil carbon

Table 2.4.22: Effect of elevated CO_2 on soil nitrogen, phosphorous, and potassium

Nitrogen (mg/Kg)						
	Control			Treated		
Treatments	CD1	CD15	% Change	TD1	TD15	% Change
T. arjuna	71.429	93.75	31.26	78.125	65.625	-12.5
S. macrophylla	80.080	98.558	23.07	86.241	117.040	35.710
P. pinnata	172.48	129.36	-25	190.960	203.281	6.45
S. glauca	98.56	117.04	18.75	129.362	104.719	-19.04
M. elengi	98.558	117.040	18.75	92.402	86.241	-6.660
S. cumini	67.759	98.558	45.45	73.920	67.759	-8.330
Phosphorous (mg/Kg)						
	Control			Treated		
Treatments	CD1	CD15	% Change	TD1	TD15	% Change
T. arjuna	39.39	43.85	11.35	42.527	51.460	-8.94
S. macrophylla	43.63	82.03	88.02	89.317	87.911	-1.57
P. pinnata	141.951	69.317	-51.16	74.246	108.406	46.31
S. glauca	35.036	37.594	7.3	34.589	25.205	-27.12
M. elengi	311.7366	224.486607	-27.98	292.870536	225.964286	-22.84
S. cumini	245.33	237.51	-3.18	225.116	248.446	10.36
Potassium (mg/Kg)						
	Control			Treated		
Treatments	CD1	CD15	% Change	TD1	TD15	% Change
T. arjuna	223.21	198.66	-10.99	267.857	251.339	-6.16
S. macrophylla	271	218	19.55	250.5	209.5	-16.36
P. pinnata	261.5	118	-54.87	222.5	155.5	-30.11
S. glauca	230	167	-27.39	273	263.5	-3.47
M. elengi	775	338	-56.38	476	364	-23.52
S. cumini	311.5	181	-41.89	356.5	236.5	-33.66

2.4.15 Correlation and PCA analysis

Correlation is carried out between the morphological, and biochemical parameters and the minerals. The correlation coefficient is significant when it is significantly different from zero. **Figure 2.4.24** represents a correlogram with only significant correlations. The blue colour indicates a positive and the red colour indicates a negative correlation. Plant height shows a positive correlation with plant pigments and carotenoids, Stem diameter is highly correlated with carbohydrates, and protein. Leaf breadth is positively correlated with plant pigments and carotenoids. Plant pigments and metabolites increase as a result of an increase in the growth characteristics of plants. Calcium is positively correlated with phenol. Magnesium is positively correlated with leaf number, stem diameter, and carbohydrates. **Figure 2.4.25** represents the biplot of PCA analysis. PCA is carried out by transforming the original data into lower dimensional space while collating highly correlated variables together. Component 1 explains 63%, component 2 explains 16% and component 3 explains 15% of total variance. In this figure component 1 is denoted by Dim 1, component 2 is denoted by Dim 2, and component 3 is three dimensional. The first principal component has high positive values for carbohydrate, protein, leaf number, and stem diameter which is represented in **Table 2.4.23**. The first principal component is strongly correlated with these variables. This suggests that these criteria vary together. Carbohydrate, protein, and leaf number are strongly correlated. Leaf breadth, plant height, carotenoids, chlorophyll a, chlorophyll b, and total chlorophyll had relatively negative values which indicates the existence of an inverse correlation between factor PCA and variables. For the second component which is denoted by the Y axis, potassium, sodium, and calcium have positive values whereas leaf area, plant height, and stem diameter have negative values. The square cosine value for each variable concerning the first 3 principal components is computed which is also shown in the biplot. Hence, it is determined how much each variable is represented in a given component. Attributes with similar square cosine scores have similar colours.

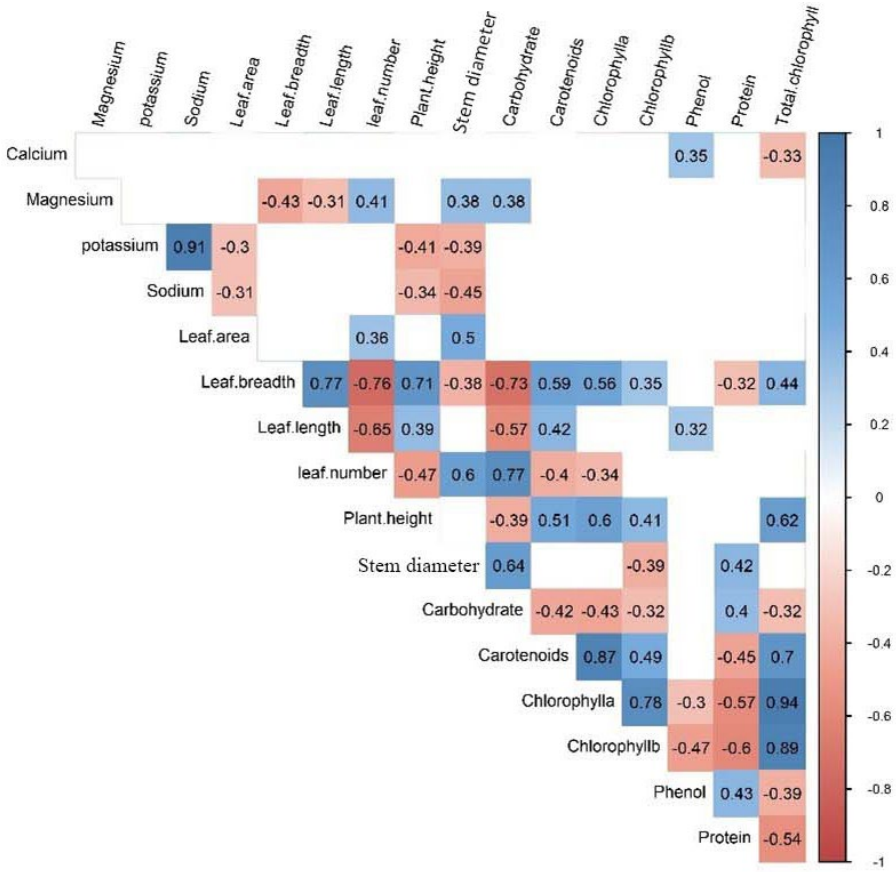


Fig: 2.4.24: Correlogram with only significant correlations

Table: 2.4.23: Loadings of each principal component

Parameters	Principal Component 1	Principal Component 2
Calcium	0.029	0.207
Magnesium	0.199	-0.112
Potassium	-0.018	0.513
Sodium	-0.049	0.516
Leaf area	0.012	-0.338
Leaf breadth	-0.335	-0.085
Leaf length	-0.198	-0.081
Leaf number	0.298	-0.136
Plant height	-0.268	-0.291
Stem diameter	0.246	-0.326
Carbohydrate	0.323	-0.101
Carotenoids	-0.306	-0.102
Chlorophyll a	-0.342	-0.131
Chlorophyll b	-0.291	0.041
Phenol	0.123	-0.036
Protein	0.264	-0.087
Total chlorophyll	-0.311	-0.160

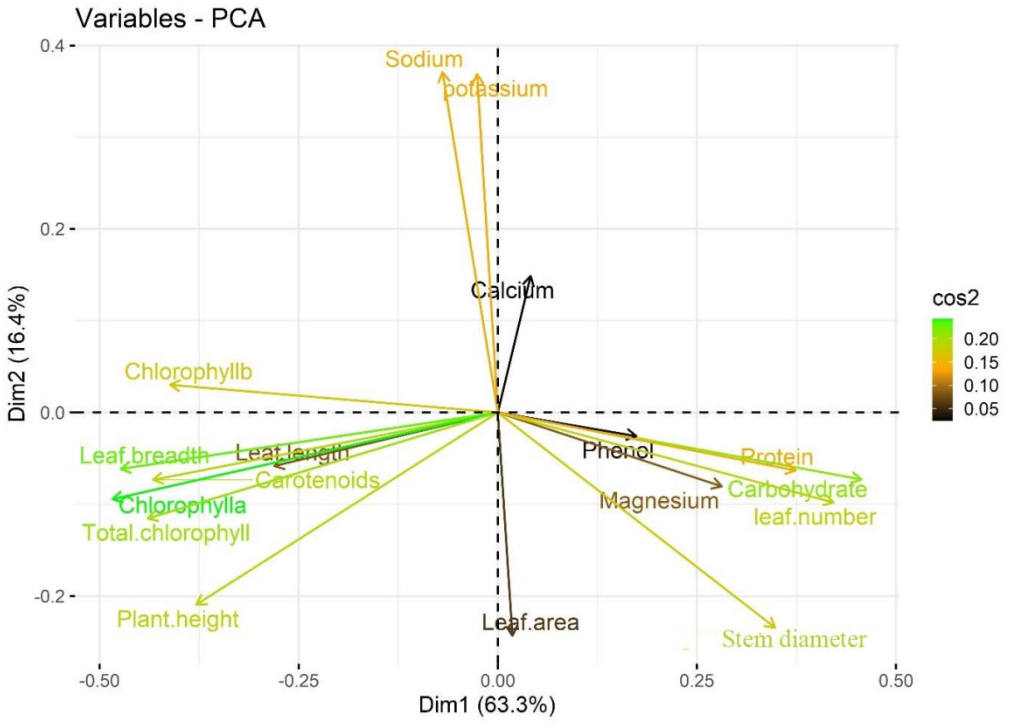


Figure 2.4.25: Biplot showing PCA analysis

2.5. DISCUSSION

As stated in Chapter I, eighteen-month-old plantlets of *Terminalia arjuna*, *Swietenia macrophylla*, *Pongamia pinnata*, *Simarouba glauca*, *Mimusops elengi*, and *Syzygium cumini* were selected separately for experimentation. For each study, 2 sets of plantlets were taken, in which one set was retained in the control chamber (CC), and the other in the treatment chamber (TC). Both the chambers were sealed from the outside. As per the methodology outlined for the standardization study (Chapter I), ambient air was pumped into the CC for about 15 minutes in the morning (9 a.m.). Similarly, the CO_2-air mixture was introduced into the TC for about 15 minutes, to ensure an elevated concentration of CO_2. The monitoring of CO_2 concentration in the CC as well as the TC was accomplished through an automated CO_2 analyzer (Fuji Electric NDIR type Infrared Gas Analyzer). Along with this, the temperature (^oC), and humidity (%) within the chambers were also monitored using a Billion Bag digital wireless electronic Hygro-thermometer.

The experiment was repeated at 6:00 p.m., with the monitoring of CO_2, temperature (^oC), and humidity (%) before and after CO_2 supplementation. The entire

experimentation was continued for 15 days. The gross and net flux of CO_2 within CC and TC and the resultant CO_2 assimilation attributed by the plants were worked out and depicted in Chapter I.

Meantime the changes in the growth and biochemical attributes associated with the plants under experimentation at various stages are monitored and are dealt with in this Chapter. The growth measurements (plant height, stem diameter, leaf length, leaf breadth, leaf number, leaf area, moisture content, and plant biomass) and biochemical estimations of plants like pigments (chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid), metabolites (carbohydrate, protein, phenol), minerals (calcium, magnesium, sodium, potassium, copper, zinc), and nutrients (carbon, nitrogen) were undertaken on days 1, 5, 10, and 15. The root measurements were undertaken on days 1 and 15. The soil characteristics studied include moisture, pH, carbon, nitrogen, phosphorus, and potassium. The discussion on the growth and biochemical responses of plants under elevated conditions of CO_2 is given below.

2.5.1 Plant height

The highest increase (%) in plant height in the TC compared to CC is observed in *T. arjuna*, followed by *P. pinnata*, *S. cumini*, *S. macrophylla*, and *M. elengi*. A slight increase in the plant height was reported in all the tree species under study, except *S. glauca* under elevated CO_2 conditions compared to the control. However, the increase in plant height was not statistically significant.

Various hypotheses support the growth of plants under specific conditions, especially at an elevated level of Carbon dioxide. Most C3 plants respond to elevated levels of CO_2 by increased net photosynthesis and resultant growth, which is relevant to the increase in height of plants (Hawkins *et al*., 2008). Increased nitrogen fixation under elevated CO_2 might support the process of growth enhancement and thereby increase in plant height (Gamper *et al*., 2005). Increased cell division under enhanced levels of CO_2 is responsible for the increase in height of plants. This is accomplished with the presence of sucrose in the meristem which helps in cell division (Ibrahim *et al*., 2018). Janani *et al*. (2016) also reported significant changes in plant morphology, including plant height and biomass in *Azadirachta indica* and *Melia dubai*, after exposure to elevated CO_2, These findings are relevant to the increased height of plants in the present study. In a CO_2-enriched environment, vegetal species acquire biomass more quickly, and thus trees take up

more carbon for growth, resulting in increased plant height (Romano and Saraiva, 2006). From the Free Air Carbon dioxide Enrichment experiment (FACE) conducted by Ainsworth and Long, (2005), it is revealed that the enhancement of plant height under elevated CO_2 is species-specific which follows the results of the present study. *T. arjuna* being a fast-growing species, performs better compared to other slow-growing ones (Zhang *et al*., 2010). Plant height of *S. glauca* has not progressed under enriched CO_2 conditions compared to control in the present study, which follows the results of Mousseau and Enoch (1989), where 2-year-old *Castanea sativa*, when exposed to elevated CO_2, resulted in an inhibition of shoot growth. This can be due to a cumulative impact, which is dependent mostly on the physiological setup of the plant, influenced by variations in external environmental conditions. Apart from this, the present study is for a short duration of 15 days, which is comparatively lesser for a tree species with a slower metabolic response to show stable growth responses.

2.5.2 Stem diameter

No statistically significant changes in the stem diameter owing to enriched CO_2 conditions are noticed with any of the species. However, a slight increase is recorded with all, except *P. pinnata*. Enhancement in stem diameter is species-specific and there are no significant changes in stem diameter in some of the deciduous tree species like *Q. mangolica*, *K. septemlobus*, *B. maximowicziana,* and *A. mono*, as reported by Watanabe *et al*., (2010). Ainsworth and Long (2005) reported an increase in stem diameter in *Populus tremuloides* under elevated CO_2. According to Pritchard *et al*. (1999), exposure to elevated CO_2 indicates a higher availability of sugar molecules for increased stem growth, which is consistent with the results of the present study, where the stem diameter has increased slightly under elevated CO_2. The results of the present study corroborate the findings of Innocente *et al*. (2020), indicating that elevated CO_2 increased the stem diameter of *E. urophylla* by stimulating cell proliferation and reducing lignin deposition. The morphological behavior of plants contributed differently to growth responses under diverse investigational environments (Singh *et al*., 2018). Elevated CO_2 drives increased stem growth by stimulating cell division within the shoot. Cell proliferation may be stimulated throughout the meristematic regions, which also accounts for an increase in nodal elongation (Pritchard *et al*., 1999). The lesser

magnitude of increase in the present study is linked with the shorter duration of exposure of the plants to elevated levels of CO_2.

2.5.3 Leaf characteristics

In the present study, leaf length showed an increase in *S. macrophylla* in TC (0.272%) compared to CC (0.0012 %) and *S. glauca* in TC (4.58%) compared to CC (0.95 %) under elevated levels of CO_2. Similarly, leaf breadth increased in *S. glauca* in TC (8.675%) compared to CC (1.337 %). Leaf number increased by 48.68 % in TC compared to CC (5.246%) and leaf area increased by 64.35 % in TC compared to CC (16.72%) in *T. arjuna*. All the above increases were statistically significant.

The results of an increased leaf area in *T. arjuna* are in line with the study of Gonzalez *et al*. (2010) where the increase in total leaf area is attributed to a change in the length/ width of the leaf of *Arabidopsis thaliana*. It can also be due to the production of an enlarged primordium during the initiation of the meristem. Increased cell division and cell expansion help in increasing the leaf area. According to Epron *et al*. (1996), leaf area of *Fagus sylvatica* increased under 2-year exposure to elevated CO_2. An increase in leaf mass per unit area is a common response to CO_2 enrichment (Mousseau and Saugier, 1992). This response is attributable to accumulations of starch or other non-structural carbohydrates (Delucia *et al*. 1985; Wong, 1990), or to an increase in leaf thickness caused by an increase in the number of palisade cell layers (Thomas and Harvey, 1983). Elevated CO_2 concentrations have been found to increase the branching, leaf area, and leaf thickness of most plants (Enoch and Zieslin, 1988). According to Taylor *et al*. (2001), the leaf area of *Populus deltoides* increased under elevated CO_2. Increased cell expansion in elevated CO_2 is associated with changes in the biophysical properties of cell walls including increased cell wall plasticity. Highly mechanistic studies of leaf growth in elevated CO_2 have been undertaken and have shown that leaf growth in elevated CO_2 is stimulated following enhanced cell expansion (Ranasinghe and Taylor, 1996) resulting from enhanced cell wall loosening and extensibility, associated with an increase in the activity of cell wall loosening enzyme xyloglucan endo-transglycosylase. The leaf length of *Glycine max* increased under elevated CO_2. The cell size of the palisade layer was also significantly affected by enriched CO_2. Enhanced CO_2 concentration increased cell area and cell perimeter mainly due to larger cell length. Carbohydrate metabolism may be involved in the regulation of

leaf cell production and leaf cell expansion. As suggested by Kinsman *et al*. (1996), and Seneweera *et al*. (1995), the activity of sucrose phosphate synthase in source leaves was enhanced with the expansion of leaf blades in elevated CO_2. The study by Taylor *et al*. (2001) aims to pinpoint the fundamental mechanisms responsible for enhanced leaf growth in elevated CO_2 and to determine whether it is valid for field-grown trees in a long-term experiment.

According to Saha *et al*. (2013), in *Cicer arietinum* (Chickpea) specific leaf area decreased under enhanced CO_2. Specific leaf area is a ratio indicating how much leaf area a plant builds with a given amount of leaf biomass. Here in chickpea, more dry matter is partitioned towards the stem which is responsible for the reduction in specific leaf area indicating lesser expansion of leaf area per unit of dry matter accumulation. Leaf area decreased in *Raphanus sativus* when exposed to elevated levels of CO_2 due to larger storage roots and decreased specific leaf area (Usuda and Shimogawara, 1998). Due to the increased accumulation of starch in plants under enhanced CO_2, the leaf area of *Quercus suber* decreased (Faria *et al*., 1996). A 38-day study using elevated CO_2 on *Prunus persicum* in the growth chamber by Davidson *et al*. (2016) also reported decreased leaf area under enhanced CO_2.

Shedding of leaves has been observed in *S. glauca* in TC. According to Aguera and De la Haba (2018), physiological and metabolic changes in plants under enhanced CO_2 levels result in increased sugar content and decreased nitrogen content in the leaves which ultimately change the C/N ratio of plants thereby triggering the leaf senescence and lead to the shedding of leaves in *S. glauca*, as noticed in the present study. Oxidative stress in plants due to enhanced CO_2 levels and decreased nitrogen has resulted in the degradation of photosynthetic pigments attributing to the senescence of leaves.

2.5.4 Plant biomass

Plant biomass increased under elevated CO_2 conditions compared to control in all the plants except *M. elengi*. The biomass enhancement ratio was calculated and found to be highest in *S. glauca* (1.45), followed by *S. macrophylla* (1.4), *S. cumini* (1.25), *T. arjuna* (1.14), *P. pinnata* (1.125), and *M. elengi* (1). It is evident from the results of the above-ground and below-ground biomass that the carbon content in *S. macrophylla* might have accumulated more in the above-ground biomass.

The mobilization of biomass to different plant organs depends to a great extent on species characteristics, ontogeny, physiological mechanisms, and the environmental conditions to which the plants are exposed (Poorter and Nagel, 2000). Light, water, nutrients, and CO_2 play an equal role in biomass allocation. In the present study, the biomass of all plants (except *M. elengi*) increased with elevated CO_2. Under enriched CO_2 conditions, fast-growing trees would have an increased metabolism or higher nutrient contents and subsequent biomass production. This was evident from the increased carbohydrate content in *T. arjuna*, and *P. pinnata*; increased carbon content in *S. glauca,* and increased nitrogen content in *S. macrophylla*. On the other hand, *M. elengi* couldn't acquire any significant enhancements in its plant metabolites and minerals under elevated CO_2 and hence no significant change was noticed in biomass (Thompson *et al*., 2017).

2.5.5 Plant moisture content

Plant moisture content increased in *P. pinnata* (4.155%) in TC compared to CC (3.86%) and it increased in *S. glauca* in TC (7.8 %) compared to a decrease of 1.39 % in CC and is statistically significant. The availability of plant moisture content to a great extent is dependent on the physiological mechanism associated with the plants. A review of elevated CO_2 effects on plant growth by Prior *et al*. (2011) revealed that the transpiration of plants decreased under elevated CO_2 and thereby increased water use efficiency. Elevated CO_2 slows transpiration by inducing partial closure of leaf stomatal guard cells (Jones and Mansfield, 1970). Elevated CO_2 induces hydraulic water conductance, changes turgor pressure inside guard cells, and increases plant moisture content (Shanker *et al*., 2022). In crops exposed to elevated concentrations of CO_2, it is observed that the stomatal conductance decreased and water use efficiency increased suggesting that water loss from plants is minimal and the moisture content is increased (Haworth *et al*., 2016). Reduction in the vapor pressure of water and CO_2 would result in improved plant water use efficiency which ultimately increases plant moisture content under enhanced CO_2 conditions (Vesala *et al*., 2017). At high CO_2 levels, reduced stomatal opening of plants helps conserve water by increasing the moisture content of the plant (Medlyn *et al*., 2001).

2.5.6 Plant pigments

In the present study, plant pigments increased under elevated CO_2 in all the plants except *M. elengi*. However, the increase is not statistically significant. The study conducted by Al-Rawahy *et al*. (2013) in *Medicago sativa* reported increased chlorophyll content under elevated levels of CO_2 due to improved substrate availability for assimilation and reduced water loss due to lower stomatal conductance.

Plant pigments such as chlorophyll a, total chlorophyll, and carotenoid decreased under elevated CO_2 conditions in *M. elengi* which is statistically significant. In *M. elengi* chlorophyll a decreased by 13.52% in TC compared to an increase of 157.43 % in CC. Chlorophyll b decreased by 8.259 % in TC compared to an increase of 171.2 % in CC. Total chlorophyll decreased by 11.20 % in TC compared to an increase of 164.09 % in CC. Carotenoids decreased by 10.55 % in TC compared to an increase of 151.05 % in CC. Plants respond to changes in their external environment by adjusting biochemically (Janani *et al*., 2016). The results of *M. elengi* follow the study of Jeong *et al*. (2018), where chlorophyll content decreased with the accumulation of non-structural carbohydrates under elevated CO_2 concentration and temperature. Open-top chamber studies conducted by Meena *et al*. (2017) reported a decrease in chlorophyll content due to temperature stress under enriched CO_2 conditions in *Capsicum annum*. Chlorophyll decline is more pronounced in plants grown under elevated CO_2 levels, which might be due to the dilution of chlorophyll and degradation by excess utilization under higher levels of CO_2 (Kumari *et al*., 2019). Faria *et al.* (1996) also reported a decrease in chlorophyll concentration in *Quercus suber* at elevated CO_2 which is explained by the dilution effect caused by the accumulation of starch. Chlorophyll reduction at elevated CO_2 can also be related to the diminution in nitrogen uptake (Delucia *et al*., 1985; Nakano *et al*., 1997). Elevated CO_2-treatment studies for 4 years in the Open-top chamber decreased the total chlorophyll concentration in *Fagus sylvatica* and *Picea abies* (Wonish *et al*., 2001). Starch accumulation was prominent in the chloroplast of *Fagus sylvatica*. According to Epron *et al*. (1996), in *Fagus sylvatica,* less nitrogen was invested in the machinery of photosynthetic light reactions, resulting in the reduction of chlorophyll content under elevated levels of CO_2. Decreased chlorophyll content under enhanced CO_2 is also reported to be due

to the inhibition of transcription of cab genes, resulting in the accumulation of soluble sugar (Van Oosten *et al*., 1994). The study conducted by Al-Rawahy *et al*. (2013) is contradictory to the above results wherein *Medicago sativa* increased chlorophyll content under elevated levels of CO_2 due to improved substrate availability for assimilation and reduced water loss due to lower stomatal conductance. Carotenoids and chlorophylls play an important role in mediating oxidative stress in plant tissues, and plants must be able to actively regulate carotenoid biosynthesis under elevated levels of CO_2 (Ormrod *et al*., 1999). In this study, temperature also plays an important role in showing the relevance of heat stress due to the elevated levels of CO_2. According to Loladze *et al*. (2019), under elevated CO_2 conditions, increased plant nutrients, water, and light efficiency are attributed to the alleviation of stress, which in turn results in the downregulation of carotenoid biosynthesis. The decline in carotenoid content in the present study might also be a stress response. Elevated CO_2 upsurges the potential for carotenoid biosynthesis in C3 plants because of the extra constituents in carotenoids (carbon, hydrogen, and oxygen), which act as precursors for carotenoid biosynthesis.

2.5.7. Plant metabolites

Carbohydrate content increased in *T. arjuna* in TC (84%) compared to CC (9.79%). However, it increased in *P. pinnata* in TC (38.20%) compared to a decrease of 24.20 % in CC and both results are statistically significant. Aguera *et al.* (2006), Hendrix *et al*. (1994), Urbonaviciute *et al*. (2006), Davidson *et al*. (2016), Ibrahim *et al*. (2018), Keutgen & Chen (2001), Faria *et al*. (1996), Ainsworth *et al*. (2002) reported higher carbohydrate content under elevated CO_2 levels in *Solanum lycopersicum, Gossypium hirsutum*, *Raphanus sativus*, *Prunus persica*, *Elaeis guineensis, Citrus limon*, *Quercus suber*, and *Glycine max*, respectively. According to Vu *et al*. (2005), in *Arachis hypogea* soluble sugar and starch increased under elevated CO_2 conditions compared to control. Usuda and Shimogawara (1998) also reported that carbohydrate content increased under enhanced levels of CO_2 in *Raphanus sativus.* A daily accumulation of starch, sucrose, and glucose increased in the study. In the present study, the increase in carbohydrates in *T. arjuna* and *P. pinnata* might be due to the non-reducing sugars which ultimately build up non-structural carbohydrates in the leaf (Janani *et al*., 2016). Carbon assimilation

resulting from the internal concentration of CO_2 in the leaf is also responsible for the increased carbohydrates.

There is no statistically significant change in the protein content under elevated CO_2, but a slight decrease is noticed in all the plants in TC compared to CC. In *T. arjuna*, *S. macrophylla,* and *S. cumini,* protein content on the final day increased compared to the first day of treatment. The results of the protein content of these plants follow the study of Sreenivasulu *et al*. (2015), wherein the protein content increased under elevated CO_2 in *Arachis hypozeae*. According to Korner *et al*. (2005), increased soil carbon provides clear evidence for the enhanced metabolic activity in soil under CO_2 enrichment, and the microorganisms present in the soil would have increased the nitrogen content in the soil, which in turn might have increased the protein concentration of the leaf. Increased protein content in plants due to an increase in the nitrogen content in the soil is also reported by Ainsworth *et al*., (2002). A study conducted in *Melia dubia* by Janani *et al*. (2016) showed a reduction in the amount of protein since the plants lost the ability to take up soil nitrate and convert it to protein at enriched levels of CO_2. Reduced protein under CO_2 enrichment was also reported by Dong *et al*. (2018) and Taub *et al.* (2008), which is in line with the results of the present study. Rubisco proteins decreased under elevated CO_2 due to the accumulation of carbohydrates serving as sugar signals and caused a decline in the photosynthetic genes when accumulated carbohydrates were not rapidly translocated or used at the sinks due to the small sink strength, which might be due to the restricted rooting capacity of plants (Li *et al*., 2008). Ainsworth and Long (2005) reported decreased protein concentration under elevated CO_2 due to decreased Rubisco content. Gifford *et al*. (2000) also suggested decreased protein and nitrogen concentrations due to the increased concentration of non-structural carbohydrates.

In the present study, the phenol content increased in *S. macrophylla* in TC (100.5%), compared to an increase of 25.46 % in CC, which is statistically significant. Increased phenol content under elevated CO_2 was reported by Ghasemzadeh *et al*. (2010) and Tognetti *et al*. (1999) in *Zingiber officinale* and *Quercus robur*, respectively. According to the CNB hypothesis, if plants increase photosynthesis and carbon gain under enriched CO_2, the excess carbon will be assigned to carbon-

based defenses, which is evident from the increased phenol content under elevated CO_2 (Bryant *et al*., 1983). In contrast to the above result, decreased phenol content in plants is associated with the carbon nutrient balance hypothesis (Coley *et al*., 2002). With nutrient fertilization, carbon can be shunted into growth resulting in decreased phenol content.

2.5.8. Minerals

In the present study, calcium decreased under enhanced CO_2 conditions by 0.453 % compared to an increase of 0.175 % in CC, and in *T. arjuna*, and in *S. cumini* an increase of 17.22 % is reported in TC compared to an increase of 20.6 % in CC. Sodium and potassium content decreased in *S. macrophylla*, *S. glauca,* and *S. cumini* in elevated CO_2 conditions compared to the control. The reduction of plant nutrients such as calcium, magnesium, potassium, and nitrogen under enriched CO_2 conditions might be due to reduced transpiration due to lower stomatal conductance (Ibrahim *et al*., 2018). Here increased humidity inside the chamber with *T. arjuna, P. pinnata, S. glauca, M. elengi,* and *S. cumini* might be due to increased transpiration of the plants. The subsequent reduction in mineral nutrients such as sodium and potassium under elevated CO_2 in these plants can be attributed to dilution by increased concentration of carbohydrates (Teng *et al*., 2006; Ibrahim *et al*., 2018; Huluka *et al*., 1994; Duval *et al*., 2012; Overdiek, 1993; Kuetgen & Chen 2001). CO_2 flux was found to be positively correlated with humidity in all the plants except *S. macrophylla* under elevated CO_2. In *S. macrophylla*, reduced transpiration of the plant resulted in decreased humidity inside the chamber and would have prevented the uptake of minerals in *S. macrophylla*. The results of Hileman *et al*. (1994) also revealed that partial closure of the stomata of cotton reduced transpiration, which in turn decreases the uptake of nutrients. Lower nutrient concentrations are caused by the accumulation of starch (Delucia *et al*., 1985; Yelle *et al*., 1989). A meta-analysis conducted by Duval *et al*. (2012) on "CO_2 effects on plant nutrient concentration" revealed that calcium, magnesium, and potassium decreased under enhanced CO_2 conditions. Elevated CO_2 reduces the nutrient movement from soil to plant roots, which is dependent on mass flow. Potassium and magnesium are essential plant nutrients that critically contribute to photosynthesis and the long-distance transport of photoassimilates. A deficiency of either potassium or magnesium decreases the CO_2 assimilation. From the study of Reddy and Zhao

(2005), it is found that cotton plants grown under elevated CO_2 were more susceptible to potassium deficiency, affecting plants. Mineral nutrients such as calcium, magnesium, sodium, and potassium in Strawberry plants decreased when exposed to elevated CO_2 (Keutgen *et al*., 1997).

The results of Meena *et al*. (2017) are in contradiction with the above statements where calcium and magnesium content in *Capsicum annuum* increased under elevated CO_2 conditions. *Cucumis sativas* when exposed to elevated CO_2 in an open-top chamber, mineral nutrients such as calcium, magnesium, and potassium increased while sodium decreased (Dong *et al*., 2018). Marinari *et al*. (2007) also reported increased magnesium content under elevated CO_2. Increased magnesium content in leaves plays a role in proton pumping ATPase which helps in phloem loading and carbohydrate partitioning.

In the present study, plant nitrogen increased under elevated CO_2 conditions in *S. macrophylla* by 9.39 % compared to a decrease of 7.35 % in CC and an increase of 5.37 % in *M. elengi* in TC compared to a decrease of 7.36 % in CC, which is in line with the results of Bunce (2016) where nitrogen content in Soybean increased under elevated CO_2 inside the open-top chamber. This could be due to the increased carbon gain of plants resulting in increased plant growth and biomass. Elevated CO_2 levels increase the uptake of nitrogen from the soil, and it changes the allocation of nitrogen towards the leaves of the plant. (Martin *et al*., 2002). Under elevated CO_2 conditions, as the photosynthesis rate increases, the plant accumulates more nitrogen for its biomass and litter resulting in a higher C: N ratio in the soil (Grover *et al*., 2015).

The results of macronutrients in the present study are contradictory to that of Teng *et al*. (2009) who reported that nitrogen content in leaves decreased under enriched CO_2 in *Arabidopsis thaliana*. Here, the dilution in nitrogen concentration may be due to the increased non-structural carbohydrates present, dilution by secondary compounds, decreased stomatal conductivity and transpiration rate, and increased nitrogen loss, which in turn results in less efficient uptake of minerals (Gifford *et al*., 2000; Mc. Donald *et al*., 2002; Riikonen *et al*., 2005; Pang *et al*., 2006). Maroco *et al*. (2002) reported decreased nitrogen content in plants when exposed to elevated CO_2 due to increased carbohydrates and increased leaf area. Plants with increased

CO_2 levels accumulate more starch, which accounts for 50% of leaf weight, ultimately leading to the depletion of other nutrients. The decrease in nitrogen concentration in elevated CO_2 could reflect higher nitrogen use efficiency leading to accelerated senescence (Stitt and Krapp, 1999; Pang *et al*. 2006; Ibrahim *et al*., 2018). Elevated CO_2 studies using open-top chambers by Meena *et al*. (2017) in *Capsicum annuum* also reported decreased nitrogen concentration when plants are exposed to elevated CO_2.

In the present study, carbon content increased in *S. glauca* in TC by 1.21 % compared to CC. The results are in agreement with the study of Melillo (1983), where carbon content in Sweetgum trees increased under elevated CO_2 conditions. In addition to this, Coley *et al*. (2002) also reported an increased C/N ratio in 9 tropical trees in the open-top chamber when exposed to elevated CO_2 whereas, in *Fagus sylvatica*, fertilization caused a significant decrease in C/N ratio because of increased nitrogen content (Lotfiomran *et al*., 2016). As nitrogen uptake is not stimulated as much as carbon uptake, CO_2 elevation alters the C/N balance in the plant body. Plants respond to elevated CO_2 by changing biomass allocation to mitigate the altered C/N balance (Kallarackal and Roby, 2012).

2.5.9 Micronutrients

Copper increased in *T. arjuna*, *S. macrophylla*, *P. pinnata, and S. cumini* under elevated CO_2 conditions. Copper increased by 10.41 % in TC in *T. arjuna* compared to a decrease of 25.91% in CC. In *S. macrophylla* copper increased by 25.49 % in *S. macrophylla* compared to a decrease of 18.47 % in CC. In *P. pinnata* an increase of 80 % in TC is reported, compared to a decrease of 14.34 % in CC. In *S. cumini* an increase of 20.46 % in TC is reported compared to a decrease of 17.97 % in CC. Zinc also increased in *T. arjuna by* 26.26 % in TC compared to an increase of 3.6 % in CC*,* In *S. macrophylla,* an increase of 35.38 % in TC was reported compared to a decrease of 36 % in CC and in *P. pinnata* an increase of 426.7% in TC was reported compared to an increase of 1.03 % in CC. Increased copper and zinc uptake in *Theobroma cacao* at higher CO_2 is due to augmented demand for mineral nutrients, which contributes to enhanced dry matter accumulations (Baligar *et al*., 2021). Elevated CO_2 levels also affect the distribution of copper and zinc in plants (Guo *et al*., 2011). A meta-analysis conducted by Duval *et al*. (2012) revealed that copper

content decreased under enhanced CO_2 conditions. *Betula pendula* when exposed to elevated CO_2 for 3 years showed a reduction in copper and zinc content (Oksanen *et al*., 2005). Copper and Zinc play an eminent role in protein production. They play an important role in the photosynthetic and respiratory electron transport chain, and cell wall metabolism. Zinc helps in ribosome development and it is an active element in the biochemical process (Seeda *et al*., 2020). Reduced stomatal conductance and water loss at elevated CO_2 levels suppress zinc content in Lettuce and Spinach (Giri *et al*., 2016).

2.5.10 FTIR analysis

The results of FTIR analysis revealed O=C=O stretching, which is an indication of the presence of the CO_2 groups in plants under elevated CO_2 conditions. The presence of Carbon dioxide is the cause of the unstructured peak in the treated sample, while the absence of Carbon dioxide is shown by the difference in the FTIR peak in the control. A prominent peak at 2360 cm^{-1} indicates the presence of CO_2 in treatment, which is absent in control.

From the study of Nandiyanto *et al*. (2019), the FTIR spectrum of carbonyl compounds such as ketones, aldehydes, esters, and carboxylic acid is represented and interpreted. From the FTIR peaks of aqueous extracts of *A. indica* by Yelmate and Thonte (2020), OH stretching, the presence of C=O (carbonyl group), C-O stretching, and C-Br stretching are reported. The presence of carbohydrates, phenol, and carotenoids in the present study is confirmed by the presence of these functional groups. The presence of alcohol, alkanes, and halogen compounds in *Erythrina variegata* is studied by Hemmalakshmi *et al.* (2017). A spectral peak at 2360 cm^{-1} corresponds to the C=O stretching of CO_2, which is strongly indicative of CO_2 (Sunila *et al.,* 2016). In the present study, FTIR peaks in the range of 1200- 900 cm^{-1} and 1700-1500 cm^{-1} indicate the presence of carbohydrates and proteins, respectively. Thus FTIR peaks provide strong evidence for the presence of CO_2 based on characteristic absorption bands it creates in the spectrum and FTIR analysis is a powerful tool for the detection and characterization of Carbon dioxide.

2.5.11 Soil characteristics

Soil has turned out to be more acidic under enhanced CO_2 conditions resulting in decreased soil pH. The percentage decrease in soil pH was more pronounced in CO_2

treated, compared to control in *T. arjuna* and *S. glauca* which is in line with the results of Smith *et al*. (2005), where a reduction in soil pH was observed under elevated CO_2. In *T. arjuna* and *S. glauca*, soil pH decreased by 18.25 % and 9.39 % in TC compared to a decrease of 11.37 % and 33 % in CC, respectively. However, the increase in soil pH under elevated CO_2 has been studied by various authors. Plant-microbe interaction is one of the causes of the increase in soil pH. As a result of an increase in the release of organic acids by plants into the soil, these exudates can enhance the activity of microbes present in soil which results in the consumption of hydrogen ions by microbes into the soil resulting in increased soil pH (Paterson *et al*., 1997).

Soil carbon has decreased in *S. macrophylla* by 16.10 % in TC compared to an increase of 30.9 % in CC. Notably, warming the soil due to increased CO_2 levels may have caused soil respiration and led to carbon loss from the soil (Dijkstra *et al*., 2012). Increased microbial decomposition of leaf litter leads to more carbon loss from the soil at elevated CO_2 conditions (Carney *et al*., 2007).

Accordingly, from the results of Korner *et al*. (2005), soil carbon concentration increased by 25 percent in elevated CO_2 as compared to the control. Jastrow *et al*. (2005) reported that elevated CO_2 increases soil carbon. Increased soil carbon storage is a result of the release of carbon to the soil, which is due to accelerated plant growth under elevated CO_2, consequent to increased photosynthesis (Rogers *et al*., 1999).

Soil moisture decreased in *T. arjuna* and *S. macrophylla* under enhanced CO_2. In *T. arjuna* soil moisture decreased by 2.957 % in TC, compared to an increase of 5.195 % in CC. In *S. macrophylla* soil moisture increased by 44. 29 % in TC compared to an increase of 51.70 % in CC. In *S. cumini* a slight increase in soil moisture is reported in TC compared to CC, which is not significant. Improved water use efficiency of C3 plants under enhanced levels of CO_2 is responsible for the increase in soil moisture under elevated levels of CO_2, which in turn resulted in enhanced CO_2 assimilation of the plant (Nelson *et al*., 2004). This increase in soil moisture is the major controlling factor in improved C assimilation rates and increased total above-ground biomass in this system (Morgan *et al*., 2001).

Raphanus sativus when exposed to elevated CO_2, resulted in deep storage roots helping in the transportation of mineral nutrients (Usuda and Shimogawara,1998). Biose *et al*. (2016) studied the soil chemical properties of *Triticum aestivum* when exposed to elevated CO_2 and found that nitrogen and phosphorous content increased and potassium content decreased under enriched CO_2 levels. The results of the present study are in agreement with the results of Biose *et al*. (2016) where soil nitrogen increased in *S. macrophylla* and *P. pinnata*, phosphorus content in *P. pinnata* and *S. cumini*, whereas potassium decreased in all the plants, both in CC and TC.

Nitrogen decreased in soil in *T. arjuna*, *S. glauca*, *M. elengi,* and *S. cumini* under enriched CO_2 conditions due to the increased nitrogen demand by plants which in turn resulted in the reduction of soil nitrogen (Koumeleh *et al*., 2007). The phosphorus in the soil increased under enriched CO_2 conditions, which might be due to the change in pH of the soil towards neutral which increased calcium and magnesium phosphates in the soil. Elevated CO_2 associated with a change in soil pH improves root and microbial exudations that increase the availability of soil plant nutrients (Koumeleh *et al*., 2007). The release of inorganic nitrogen to the soil solution is more for a plant grown under elevated CO_2 conditions. Long-term CO_2 enrichment studies are effective in studying the effects of elevated CO_2 on soil since soil carbon sequestration is a long-term process (Brett *et al*., 2009). Positive shifts in root system growth altered soil structural characters (Prior *et al*., 2004).

On an overall assessment of the growth and biochemical attributions of plants due to a higher level of Carbon dioxide supply, it has been noticed that *T. arjuna* and *S. macrophylla* can be promoted for carbon offset planting due to their increased CO_2 assimilation, lower respiratory release, increased growth attributes like leaf length in *S. macrophylla*, and increased leaf number and leaf area in *T. arjuna.* Increased carbohydrate content in *T. arjuna* and *P. pinnata* makes them significant in the perspective of Carbon sequestration. However, higher respiratory release, lower biomass production, and lower CO_2 assimilation, compared to *S. macrophylla* and *T. arjuna* make *P. pinnata* next only to the latter in their efficiency for Carbon sequestration. Even though higher biomass and higher leaf length are reported in *S.*

glauca under elevated CO_2 conditions, lower Carbon dioxide assimilation and higher respiratory release by the plant make them poor candidates for carbon sequestration. Also, low Carbon dioxide assimilation and biomass production by *M. elengi*, low Carbon dioxide assimilation, and higher respiratory release by *S. cumini* make them next only to *S. macrophylla*, and *T. arjuna* in the process of Carbon assimilation.

2.6 SUMMARY AND CONCLUSION

In continuation to Chapter I, the present Chapter deals with an evaluation of the growth and biochemical responses of *Terminalia arjuna*, *Swietenia macrophylla*, *Pongamia pinnata*, *Simarouba glauca*, *Mimusops elengi*, and *Syzygium cumini* subjected to an elevated level of CO_2. Variations in morphological characteristics such as plant height, stem diameter, leaf length, leaf breadth, leaf number, and leaf area under control and elevated CO_2 conditions were compared. Also, variations in plant pigments such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids and percentage changes in metabolites (carbohydrate, protein, and phenol), and minerals (calcium, magnesium, sodium, and potassium) under control and enriched CO_2 conditions were studied. CHNS analysis was carried out to determine the changes in plant carbon and nitrogen under enhanced CO_2 conditions. Variations in copper and zinc were obtained using Atomic Absorption Spectroscopy (AAS). Plant moisture content under control and elevated CO_2 conditions were compared. Changes in the functional groups were analyzed using FTIR studies. Variations in soil characteristics such as soil moisture, soil pH, soil carbon, soil nitrogen, phosphorous and potassium under control and elevated CO_2 conditions were also compared.

Leaf length increased in *S. macrophylla* and *S. glauca* under elevated CO_2 conditions compared to control, whereas leaf breadth increased only in *S. glauca*. Leaf number and leaf area increased in *T. arjuna* under enriched CO_2 conditions. Significant differences in plant height, leaf length, leaf breadth, leaf number, and leaf area between the treated plants were noticed. Biomass of all the plants increased

under elevated CO_2 conditions except *M. elengi* and was highest in *S. glauca* followed by *S. macrophylla*, *S. cumini*, *T. arjuna*, and *P. pinnata*.

In *M. elengi*, the amount of carotenoid and chlorophyll dropped with an increased CO_2 supply, indicative of an initial stressful condition. The higher levels of carbohydrates in *T. arjuna* and *P. pinnata* under elevated CO_2 supply can result from an increase in carbon absorption brought about by the internal concentration of CO_2 in the leaf. The higher phenol content in *S. macrophylla* may be due to an excess carbon being used for carbon defenses under situations of enriched CO_2. Significant variations in calcium were seen between *S. cumini* and *T. arjuna* in both control and elevated CO_2 environments. Sodium and potassium levels were reduced in *S. macrophylla*, *S. glauca,* and *S. cumini* under higher levels of CO_2. Reduction in potassium in TC can be attributed to the increased stomatal conductance or increased carbohydrate dilution. Under conditions of elevated CO_2, there is a reported rise in plant nitrogen in *S. macrophylla* and *M. elengi* and a decrease in *T. arjuna*, *P. pinnata*, and *S. glauca*. The carbon content in *S. macrophylla*, and *S. glauca* increased under elevated levels of CO_2. Additionally, FTIR studies suggest that under high CO_2 conditions, plants consume CO_2. A positive correlation is seen between the morphological traits of plants, plant metabolites, and minerals based on Pearson's correlation coefficient. Soil carbon decreased in *S. macrophylla*, whereas soil nitrogen increased in *S. macrophylla* and *P. pinnata*. Following the supply of CO_2, phosphorus concentration increased in soils containing *P. pinnata*, and *S. cumini.*

The results of the present study suggest that elevated Carbon dioxide concentration had varied degrees of influence on the growth and biochemical characteristics of the plants under study.

Increased growth and biochemical characteristics of *S. macrophylla* reveal that the plant assimilates more Carbon dioxide compared to the other plants. Increased leaf length, phenol content, plant carbon, and nitrogen in *S. macrophylla* are indicative of their efficiencies of carbon sequestration. However, minerals such as sodium and potassium decreased in *S. macrophylla* under elevated CO_2 conditions.

The assimilation of CO_2 by *T. arjuna* is evident from the increased leaf number, leaf area, and carbohydrate content. Similarly, the assimilation of CO_2 by *P. pinnata* is

evident from the increased carbohydrate content of the plant. Consumption of CO_2 by *S. glauca* is evident from the increased leaf length, leaf breadth, plant carbon, and plant biomass. Plant nitrogen increased in *M. elengi* under elevated CO_2. Minerals such as calcium, sodium, and potassium decreased under elevated CO_2 in *S. cumini*. Thus, considering the growth and metabolic responses, species like *S. macrophylla, T. arjuna, P. pinnata*, and *S. glauca* can be recommended for carbon offset planting, aiming at Carbon dioxide sequestration.

GENERAL CONCLUSIONS

The present study revealed that CO_2 assimilation is higher in *S. macrophylla*, followed by *T. arjuna*, *P. pinnata*, *S. glauca*, *S. cumini*, and *M. elengi*. The temperature and day flux in CO_2 are shown to be negatively correlated in all the plants except *S. macrophylla*. CO_2 flux and humidity are found to be positively correlated in all the plants except *S. macrophylla.* The correlation between temperature and humidity is found to be negative in all the plants under elevated CO_2 conditions. All the above correlations are statistically significant. Considering the performances of the plants under study concerning the day and night fluxes, it has been noticed that *S. macrophylla*, followed by *T. arjuna*, are effective in carbon assimilation, with higher accumulation during daytime and lower releases during nighttime. *P. pinnata* and *S. glauca*, though significant in terms of daytime assimilation of Carbon dioxide, their resultant release during nighttime was higher, showing lesser performances in the perspective of Carbon sequestration.

Increased growth and biochemical characteristics of *S. macrophylla* reveal that the plant consumes more carbon dioxide compared to the other plants. Increased leaf length, phenol content, plant carbon, and nitrogen in *S. macrophylla* indicate the efficiency of the plant in carbon sequestration. Minerals such as sodium and potassium decreased in *S. macrophylla* under enriched CO_2. Consumption of CO_2 by *T. arjuna* is evident from the increased leaf number, leaf area, and carbohydrates. Consumption of CO_2 by *P. pinnata* is evident from the increased carbohydrate content of the plant. Consumption of CO_2 by *S. glauca* is evident from the increased leaf length, leaf breadth, plant carbon, and plant biomass. Thus, out of 6 plants *S. macrophylla, T. arjuna, P. pinnata, S. glauca* show increased growth characteristics and plant metabolites under elevated CO_2 conditions.

The present study reveals that the CO_2 assimilation potentials of *S. macrophylla* are higher, followed by *T. arjuna*, *P. pinnata*, *S. glauca*, *S. cumini*, and *M. elengi*. The species *S. macrophylla* is found to be more efficient in carbon sequestration, due to its increased CO_2 assimilation, lower respiratory release, increased biomass content, increased growth characteristics, metabolites, and nutrients.

ANNEXURE

Annexure 1: Wilcoxon Signed rank test for CO_2 flux of plants under elevated CO_2

Species	v	Sig
Terminalia arjuna	105	<0.001
Swietenia macrophylla	105	<0.001
Pongamia pinnata	105	<0.001
Simarouba glauca	105	<0.001
Mimusops elengi	105	<0.001
Syzygium cumini	105	<0.001

Annexure 2: Wilcoxon Signed rank test for the changes in daytime temperature between CC and TC

Species	v	Sig
Terminalia arjuna	31	0.556
Swietenia macrophylla	6	0.003
Pongamia pinnata	14	0.017
Simarouba glauca	10	0.014
Mimusops elengi	8.5	0.006
Syzygium cumini	0	0.001

Annexure 3: Wilcoxon Signed rank test for the changes in nighttime temperature between CC and TC

Species	v	Sig
Terminalia arjuna	85	0.044
Swietenia macrophylla	97.5	0.005
Pongamia pinnata	71	0.258
Simarouba glauca	71	0.08
Mimusops elengi	99	0.003
Syzygium cumini	105	0.001

Annexure 4: Two sample t test for the changes in the morphological characteristics of plants between CC and TC

Plant height			
Species	t	df	Sig
Terminalia arjuna	-1.381	6	0.216
Swietenia macrophylla	-0.243	6	0.815
Pongamia pinnata	-1.258	6	0.254
Simarouba glauca	1.091	6	0.317
Mimusops elengi	-0.707,	4	0.518
Syzygium cumini	-1.941	4	0.124
Stem diameter			
Species	t	df	Sig
Terminalia arjuna	-0.48	6	0.648
Swietenia macrophylla	-0.034	6	0.973
Pongamia pinnata	1.457	6	0.195
Simarouba glauca	-1.002	6	0.3546
Mimusops elengi	-0.304	4	0.775
Syzygium cumini	-1.732	4	0.158
Leaf length			
Species	t	df	Sig
Terminalia arjuna	1.014	6	0.349
Swietenia macrophylla	-3.137	6	0.02
Pongamia pinnata	0.954	6	0.376
Simarouba glauca	-4.072	6	0.006
Mimusops elengi	0.276	4	0.795
Syzygium cumini	-0.443	4	0.6802
Leaf breadth			
Species	t	df	Sig
Terminalia arjuna	-0.340	6	0.744
Swietenia macrophylla	-0.51	6	0.627
Pongamia pinnata	2.158	6	0.074
Simarouba glauca	-2.979	6	0.024
Mimusops elengi	-0.165	4	0.876
Syzygium cumini	0.657	4	0.546
Leaf number			
Species	t	df	Sig
Terminalia arjuna	-3.732	6	0.009
Swietenia macrophylla	-1.005	6	0.353
Pongamia pinnata	-0.997	6	0.357
Simarouba glauca	0.398	6	0.703
Mimusops elengi	-0.106	4	0.92
Syzygium cumini	0.133	4	0.90

Leaf area			
Species	t	df	Sig
Terminalia arjuna	-4.115	6	0.006
Swietenia macrophylla	-2.143	6	0.075
Pongamia pinnata	-0.445	6	0.671
Simarouba glauca	0.099	6	0.924
Mimusops elengi	0.043	4	0.967
Syzygium cumini	0.01	4	0.991

Annexure 5: Kruskal Wallis test for the changes in the morphological characteristics between plants

Morphological characters	H	df	Sig
Plant height	13.388	5	0.02
Stem diameter	6.313	5	0.276
Leaf length	5.187	5	0.393
Leaf breadth	15.767	5	0.007
Leaf number	11.437	5	0.043
Leaf area	14.095	5	0.015

H- Kruskal wallis chi square

Annexure 6: Two sample t test for the changes in the plant pigments in CC and TC

Chlorophyll a			
Species	t	df	Sig
Terminalia arjuna	0.84	6	0.433
Swietenia macrophylla	0.153	6	0.883
Pongamia pinnata	-0.351	6	0.737
Simarouba glauca	0.445	6	0.671
Mimusops elengi	4.913	4	0.007
Syzygium cumini	1.869	4	0.134
Chlorophyll b			
Species	t	df	Sig
Terminalia arjuna	0.266	6	0.798
Swietenia macrophylla	0.788	6	0.46
Pongamia pinnata	-1.658	6	0.148
Simarouba glauca	0.945	6	0.381
Mimusops elengi	4.899	4	0.008
Syzygium cumini	-1.827	4	0.141
Total chlorophyll			
Species	t	df	Sig
Terminalia arjuna	0.404	6	0.699
Swietenia macrophylla	-0.056	6	0.957
Pongamia pinnata	-0.515	6	0.624
Simarouba glauca	0.603	6	0.568
Mimusops elengi	4.914	4	0.007
Syzygium cumini	0.650	4	0.55

Carotenoids			
Species	t	df	Sig
Terminalia arjuna	1.017	6	0.348
Swietenia macrophylla	0.641	6	0.545
Pongamia pinnata	-0.807	6	0.450
Simarouba glauca	0.607	6	0.565
Mimusops elengi	5.458	4	0.005
Syzygium cumini	0.387	4	0.718

Annexure 7: Kruskal Wallis test for the changes in the pigments among plants

Plant pigments	H	df	Sig
Chlorophyll a	13.246	5	0.021
Chlorophyll b	14.565	5	0.012
Total chlorophyll	17.267	5	0.004
Carotenoids	17.466	5	0.003

Annexure 8: Two sample t test for the changes in the plant metabolites in CC and TC

Carbohydrate			
Species	t	df	Sig
Terminalia arjuna	1.017	6	0.348
Swietenia macrophylla	-0.652	6	0.538
Pongamia pinnata	-2.558	6	0.042
Simarouba glauca	2.022	6	0.089
Mimusops elengi	-1.328	4	0.254
Syzygium cumini	1.376	4	0.24
Protein			
Species	t	df	Sig
Terminalia arjuna	0.972	6	0.368
Swietenia macrophylla	1.684	6	0.143
Pongamia pinnata	-0.081	6	0.937
Simarouba glauca	0.314	6	0.763
Mimusops elengi	0.740	4	0.50
Syzygium cumini	2.393	4	0.074
Phenol			
Species	t	df	Sig
Terminalia arjuna	0.743	6	0.485
Swietenia macrophylla	-7.693	6	<0.001
Pongamia pinnata	-0.904	6	0.40
Simarouba glauca	-0.412	6	0.694
Mimusops elengi	0.789	4	0.473
Syzygium cumini	1.11	4	0.328

Annexure 9: Kruskal Wallis test for the changes in the metabolites among plants

Plant metabolites	H	df	Sig
Carbohydrate	13.494	5	0.019
Protein	16.911	5	0.004
Phenol	16.068	5	0.006

Annexure 10: Two sample t test for the changes in plant minerals in CC and TC

Calcium			
Species	t	df	Sig
Terminalia arjuna	6.397	6	<0.001
Swietenia macrophylla	-0.709	6	0.504
Pongamia pinnata	-1.571	6	0.167
Simarouba glauca	1.476	6	0.19
Mimusops elengi	2.405	4	0.073
Syzygium cumini	2.619	4	0.058
Magnesium			
Species	t	df	Sig
Terminalia arjuna	0.042	6	0.967
Swietenia macrophylla	2.012	6	0.09
Pongamia pinnata	-0.187	6	0.857
Simarouba glauca	0.271	6	0.794
Mimusops elengi	-1.302	4	0.262
Syzygium cumini	-1.276	4	0.270
Sodium			
Species	t	df	Sig
Terminalia arjuna	3.847	6	0.008
Swietenia macrophylla	2.283	6	0.062
Pongamia pinnata	- 0.615	6	0.561
Simarouba glauca	19.264	6	0.001
Mimusops elengi	1.926	4	0.126
Syzygium cumini	4.414	4	0.011
Potassium			
Species	t	df	Sig
Terminalia arjuna	1.869	6	0.110
Swietenia macrophylla	-0.690	6	0.515
Pongamia pinnata	3.364	6	0.015
Simarouba glauca	3.288	6	0.016
Mimusops elengi	0.564	4	0.602
Syzygium cumini	727.46	4	0.002

Annexure 11: Kruskal Wallis test for the changes in plant minerals among plants

Plant minerals	H	df	Sig
Calcium	4.832	5	0.436
Magnesium	10.984	5	0.051
Sodium	18.291	5	0.002
Potassium	20.099	5	<0.001

Annexure 12: Wilcoxon rank sum exact test for the changes in soil characters in CC and TC

Soil moisture		
Species	w	Sig
Terminalia arjuna	1	0.05
Swietenia macrophylla	1	0.05
Pongamia pinnata	8	1
Simarouba glauca	12	0.342
Mimusops elengi	0	0.1
Syzygium cumini	1	0.2
Soil pH		
Species	w	p
Terminalia arjuna	14	0.114
Swietenia macrophylla	4	0.342
Pongamia pinnata	13	0.2
Simarouba glauca	16	0.028
Mimusops elengi	3	0.7
Syzygium cumini	5	1
Soil carbon		
Species	w	p
Terminalia arjuna	2.5	0.146
Swietenia macrophylla	5	0.465
Pongamia pinnata	8.5	1
Simarouba glauca	10	0.685
Mimusops elengi	3	0.7
Syzygium cumini	4	1

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