



Growth, physiological and biochemical responses of Meliaceae species - *Azadirachta indica* and *Melia dubia* to elevated CO₂ concentrations

S. Janani, P. Priyadharshini, R. S. C. Jayaraj, C. Buvaneswaran, Rekha R. Warriar*

Institute of Forest Genetics and Tree Breeding, P.B.No.1061, R.S.Puram, Coimbatore-641002, India.

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ABSTRACT

Response of two important tropical tree species of Meliaceae (*Azadirachta indica* – neem and *Melia dubia* – melia) to elevated levels of CO₂ (600 and 900 ppm) under simulated temperature and moisture regimes in Automated Open Top Chambers was studied. Growth, biochemical changes, antioxidant property and gas exchange parameters were estimated. The results indicate that *A. indica* is expected to acclimatize under elevated CO₂ concentrations whereas *M. dubia* was observed to be a species sensitive to elevated CO₂ concentrations, affecting the photosynthetic machinery, stomatal conductance and transpiration and a subsequent decrease in carbohydrates, proteins, sugars, amino acids and phenolics. The short-term and long-term responses with respect to stomatal conductance and transpiration rates were higher in neem than melia. Thus, a positive response of neem to increased CO₂ concentrations is a good indication for its future establishment in potentially changed climatic conditions.

1. INTRODUCTION

Plants acclimatise and adapt to the prevailing environmental conditions to optimize their growth and survival. It is well demonstrated that the growth and morphology related responses of plants to elevated atmospheric carbon dioxide concentration vary according to species and are dependent on the environmental conditions [1]. Different species respond differently to elevated carbon dioxide. Most plants growing in experimental environments with increased levels of atmospheric carbon dioxide exhibit increased rates of net photosynthesis and reduced stomatal conductance, resulting in increased productivity [2] and an enhancement in water-use efficiency [3]. Stimulation of carbon assimilation resulting from increased internal CO₂ concentrations in enriched environment leads to increased stem and foliar biomass [4]; greater root biomass and volume [5]; and stimulation of root symbiotic associations [6,7]. The effect of elevated CO₂ on net photosynthesis in temperate trees has been well documented [8,9]. Net photosynthesis in loblolly pine trees and seedlings has been demonstrated to be boosted by both short and long term exposure to elevated CO₂.

The effect of elevated CO₂ on tree biomass production has been well characterized. In general, under optimum growing conditions (i.e. adequate nutrients, water and light), long term exposure to elevated CO₂ (approximately double ambient CO₂ for one season or more) can increase biomass by up to 130% in conifer and 49% in deciduous trees [10]. On an average, biomass production was stimulated by approximately 31% in loblolly pine grown in elevated CO₂ [11].

The combined effect of elevated CO₂ and elevated temperature may have an interactive effect on Anet. While fewer studies have investigated the effect of both elevated CO₂ and temperature, there are reports of both positive and negative responses in different studies. In mature *Picea abies* ((L.) Karst.) [12] and in seedlings of loblolly pine there was an increase in the optimum temperature for Anet in trees grown under elevated CO₂. Contrarily, mature *Pinus sylvestris* (L.) [13] and *Betula papyrifera*, *Picea mariana* and *Populus tremuloides* [14] observed a decrease in Anet with an increase in temperature under both ambient and elevated CO₂ suggesting that there was no interactive effects of temperature by CO₂ treatment interaction. The present study was taken up to investigate the biochemical responses of two tropical tree species of family Meliaceae to elevated CO₂.

* Corresponding Author

Tel: 91 422 2484167; Fax: 91 422 2430549

Mail id: rekhawarrier@gmail.com

The two species selected for the study are commercially important in the family Meliaceae - *Azadirachta indica* (neem) and *Melia dubia* (melia). The former, popular for its timber and medicinal principles is slow growing while the latter, which finds use in the ply industry shows an MAI (Mean Annual Increment) of ~ 0.25 m per year.

Our principal objective in this study was to investigate the variations in growth responses to elevated CO₂ in tropical tree species of the same family. A second objective was to investigate responses of gas exchange parameters and biochemicals to elevated temperature and CO₂ in the two tropical tree seedlings neem and melia. We aimed to test the hypothesis: growth responds more strongly to elevate CO₂ in the slow growing neem than in the fast growing *Melia* seedlings grown in unfertilized soil.

2. MATERIALS AND METHODS

Seedlings of *Melia dubia* Cav. and *Azadirachta indica* A. Juss were subjected to germination. Uniform seedlings of the experimental species approximately 10 cm tall were selected and transplanted into bags (30 x 25 cm). The initial biomass was measured on seedlings of the same size. The pots were filled with equal proportions of river sand, farmyard manure and soil (2:1:1). All plants were watered each morning to saturation.

All plants were grown in four chambers, of dimensions 3 x 3 x 3 m, fabricated with galvanized iron (GI) pipe frames and covered with polyvinyl chloride (PVC) sheet of 120 μ gauge to have more than 90% transmittance of light with top open to maintain near-natural conditions. A software facility called Supervisory Control and Data Acquisition (SCADA) was used to continuously control, record and display the actual and desired CO₂ level, relative humidity and temperature in each OTC by feedback control loop passing through Programmable Logical Controllers (PLC). The CO₂ enrichment was done to maintain the level of concentrations (600 ppm and 900 ppm) using a CO₂ cylinder, on a daily basis and the CO₂ levels were monitored through SCADA and PLC. The experimental design used was randomized complete block design (RBD) with five replications. The treatments were:

- Treatment 1- Control chamber in order to study the effect of chamber environment (CO₂ concentration maintained same as the ambient i.e. 380 ppm).
- Treatment 2 - 600 \pm 50 ppm level of CO₂
- Treatment 3 - 900 \pm 50 ppm level of CO₂
- Treatment 4 - 900 ppm level of CO₂ maintaining a temperature of ambient + 4°C (using humidifier)
- Control – Ambient conditions (prevailing CO₂ levels - 380 ppm) with no chambers

There were 4 CO₂ treatments, 2 plant species 5*5 replicate plants = 200 total. In three chambers, air temperature was maintained at 30/22°C (day/night) with a photoperiod of 16 h; relative humidity was ca. 50% and photosynthetic photon flux density (PPFD) was ca. 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the day. The CO₂ treatment lasted from 8th December to 12th April (125 days).

2.1 Measurements of Leaf gas exchange

Leaf gas exchange was measured with Li-6400 (Li-Cor, Lincoln, NE, USA). The net photosynthetic rate (Pn), transpiration rates (E), stomatal conductance (gs) and internal carbon-di-oxide concentration (Ci) were measured on fully expanded leaves to evaluate the effect of elevated CO₂. All these measurements were recorded in twenty five plants per species and three replications per leaf 125 days after the seedlings were exposed to elevated CO₂ concentrations. The measurements were performed at midday, between 1000 and 1200 eastern daytime, when the solar photosynthetic photon irradiance was saturated at 1000-1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under cloud free conditions. The photosynthetic chamber was calibrated at regular intervals using two reference CO₂ cylinders before taking the measurements. After the reference CO₂ concentration become stabilized (~5 min), the chamber CO₂ was matched to the reference CO₂ so that the chamber and reference CO₂ concentrations were equilibrated prior to a leaf being inserted into the chamber. The leaf was allowed to equilibrate for 5 min before measurements were taken and the duration of each measurement was usually 30-45 seconds. To minimize the heat load within the cuvette, a small external fan was used to control temperature. The relative humidity at the inlet of the cuvette was kept between 60 and 80%.

Water use efficiency (WUE) was calculated using net photosynthetic rate and transpiration (Pn/E). Intrinsic water use efficiency (IWUE) was calculated as the ratio of the net photosynthetic rate to the stomatal conductance (Pn/g_s). Intrinsic carboxylation efficiency (ICE) was derived as the ratio of the net photosynthetic rate of intercellular CO₂ concentration (Pn/Ci). Intrinsic Mesophyll Efficiency (IME) was derived as the ratio of intercellular CO₂ concentration to the stomatal conductance (Ci/g_s).

The long-term response of g_s was quantified as the ratio of g_s for plants grown at the elevated CO₂ to those of plants grown at the ambient CO₂, both variables measured at a standard CO₂ concentration of 380 ppm. The direct or short-term effect (STE) of increasing CO₂ concentration was quantified as the ratio of stomatal conductance (g_s) measured at elevated CO₂ concentration to the plant grown at the ambient growth conditions [15]. A similar ratio was calculated for the net CO₂ assimilation rate (NAR), which was modified [16].

2.2 Growth and Biochemical Measurements

All plants were harvested and sorted into leaves, stems and roots at the end of the growth period. Roots, leaves, and stems were separated and dry weights were determined after 48 h in an oven set at 70 °C. From the basic data on fresh weight and dry weight of root, shoot and leaves, derived parameters such as Root-Shoot allometric ratio (RSR) and percent of total biomass (TB) allocated to various plant organs (LBP- Leaf Biomass Portioning; SBP- Shoot Biomass Portioning; RBP- Root Biomass Portioning) was calculated. Leaves were collected from the two tree species from each ambient and elevated OTCs. All samples were collected from fully expanded leaves at 8:00 h. The samples were

immediately stored in -20 °C, and kept until analysis. Estimation of chlorophyll [17], protein [18], carbohydrate [19], reducing sugars [20], free amino acids [21], phenols [22], flavanoids [23]; DPPH scavenging assay [24], carbonic anhydrase [25] were carried out as described.

2.3 Data analysis

Effects of CO₂ concentration, species, and their interaction on variables measured in this study were evaluated using analysis of variance (ANOVA) with SPSS statistics package. The comparison of means was conducted using the Duncan's Multiple Range Test (DMRT) where ANOVA showed a significant effect. Students' T test was performed to compare the effects of CO₂ on performance of seedlings. Simple correlations were carried out with parameters which showed significant variations at interaction and treatment levels. Prior to statistical analyses, variables were checked for normality and transformed when necessary [26].

3. RESULTS

3.1 Measurements of Leaf gas exchange

Table 1a presents the significance levels for photosynthetic and gas exchange characteristics both at species and treatment levels and their interactions. Treatment levels and their interactions were found to be insignificant for all derived parameters namely WUE, IWUE, ICE and IME. At the species level, they varied significantly with respect to the different parameters assessed except Ci and IWUE. Various elevated CO₂

treatments affected gas exchange characteristics but did not affect Pn. Interactive effects were significant only for transpiration and stomatal conductance.

Figure 1 presents the percent increase/decrease in response of the two species with respect to control subjected to different treatments. Gas exchange characteristics in neem increased in response to different treatments. In melia, Pn and gs showed an increase while Ci and E recorded a decrease. The increase in Pn, gs and E of neem was very high (>75 per cent) when compared to the corresponding increase in melia (<25 per cent) which was almost near normal values.

Among the derived physiological parameters, neem and melia showed increased response to all except IME. Contrarily, neem showed decreased response in the case of ICE. Overall, the response of melia was near normal in comparison to neem which showed wide variability.

Stomatal conductance showed an increase under short and long term exposure of neem with about 192 percent increase for short term and a reduction from the same in the long term (130 percent). A similar trend was observed towards photosynthetic responses also with Pn values showing an increase by 120 and 63 percent under short term and long term responses respectively (Table 2). Melia recorded low values with respect to short-term responses of stomatal conductance and photosynthetic responses (both non-significant at 5 per cent levels). However, the long-term responses increased for both parameters recording values of 11 and 21 per cent respectively which were significantly higher (Table 2).

Table 1a: Significance level (F value) of effects of different factors and factor interactions on physiological traits based on two-way ANOVA.

Variables	Species	Treatments	Species x Treatment
Net Photosynthetic Rate (Pn)	29.766**	0.657ns	0.771ns
Stomatal Conductance (gs)	155.907**	9.284**	8.974**
Intercellular CO ₂ Concentration (Ci)	3.828ns	1.41**	1.674ns
Transpiration Rate (E)	254.931**	6.22**	6.393**
Intrinsic Water Use Efficiency (WUE)	5.487**	0.613ns	1.616ns
Instantaneous Water Use Efficiency (IWUE)	1.283ns	1.298ns	2.515ns
Intrinsic Carboxylation Efficiency (ICE)	7.42**	0.263ns	0.246ns
Intrinsic Mesophyll Efficiency (IME)	24.522**	0.175ns	0.231ns

Significance levels: ns, p>0.05; *p<0.05; **p<0.01

Table 1b: Significance level (F value) of effects of different factors and factor interactions on morphological traits based on two-way ANOVA.

Variables	Species	Treatments	Species x Treatment
Shoot length	20.089**	24.739**	1.562ns
Number of Leaves	28.85**	0.601ns	0.219ns
Number of Roots	27.692**	1.681ns	0.519ns
Root length	10.067**	0.28ns	0.781ns
Root Shoot Ratio	3.723ns	2.876ns	0.851ns
Total Biomass	1.071ns	7.294**	0.333ns
Root Biomass Partitioning	7.205**	2.727ns	1.969ns
Shoot Biomass Partitioning	15.27**	3.603**	0.953ns
Leaf Biomass Partitioning	4.046ns	3.619**	1.531ns

Significance levels: ns, p>0.05; *p<0.05; **p<0.01

Table 1c: Significance level (F value) of effects of different factors and factor interactions on biochemical traits based on two-way ANOVA.

Variables	Species	Treatments	Species x Treatment
Free amino acids	10196.2**	654.5**	544.8**
Total Carbohydrates	3097.8**	44.8**	55.1**
Chlorophyll a	81.5**	11.1**	33.7**
Chlorophyll b	6.5**	30.9**	18.6**
Total Chlorophyll	24.2**	17.1**	11.6**
Total Proteins	515.4**	651.7**	482.7**
Free Reducing Sugars	537.3**	64.7**	697.1**
DPPH antioxidant assay	6991.9**	317.2**	23.7**
Flavonoids	7088.1**	466.3**	629.4**
Total phenolics	420.1**	71.9**	68.0**
Tannins	270.7**	144.7**	48.2**
Carbonic Anhydrase activity	7991.6**	747.6**	987.6**

Significance levels: ns, p>0.05; *p<0.05; **p<0.01.

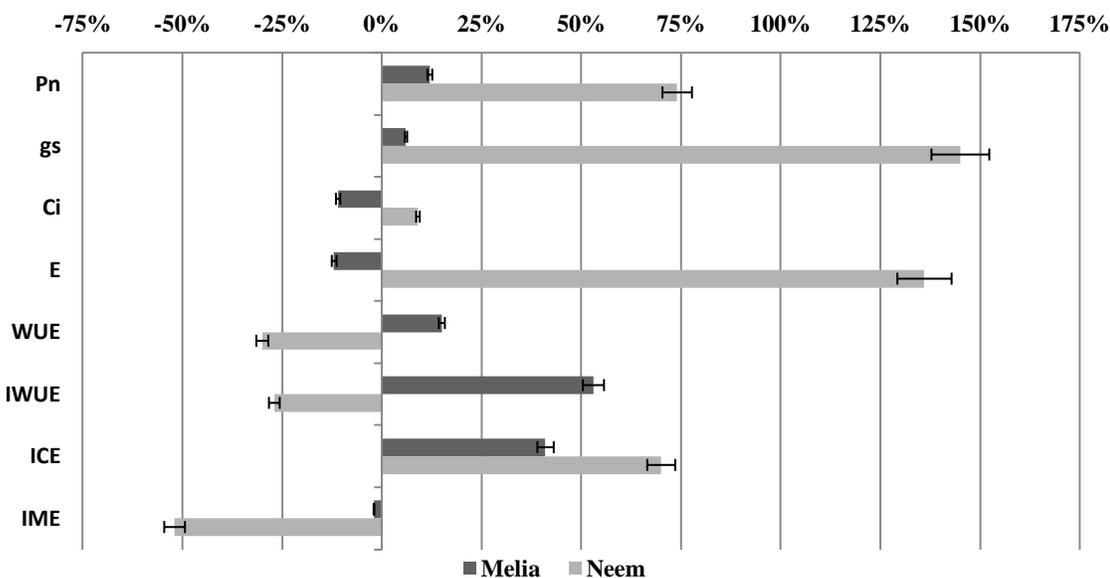


Fig. 1: The average increase (%) in physiological parameters under chamber, elevated (600 ppm, 900 ppm and 900 ppm with RH) relative to ambient (380 ppm) CO₂ for melia (dark shaded bars) and neem (light shaded bars). *Net Photosynthetic Rate (Pn)*, *Stomatal Conductance (gs)*, *Intercellular CO₂ Concentration (Ci)*, *Transpiration Rate (E)*, *Intrinsic Water Use Efficiency (WUE)*, *Instantaneous Water Use Efficiency (IWUE)*, *Intrinsic Carboxylation Efficiency (ICE)*, *Intrinsic Mesophyll Efficiency (IME)*.

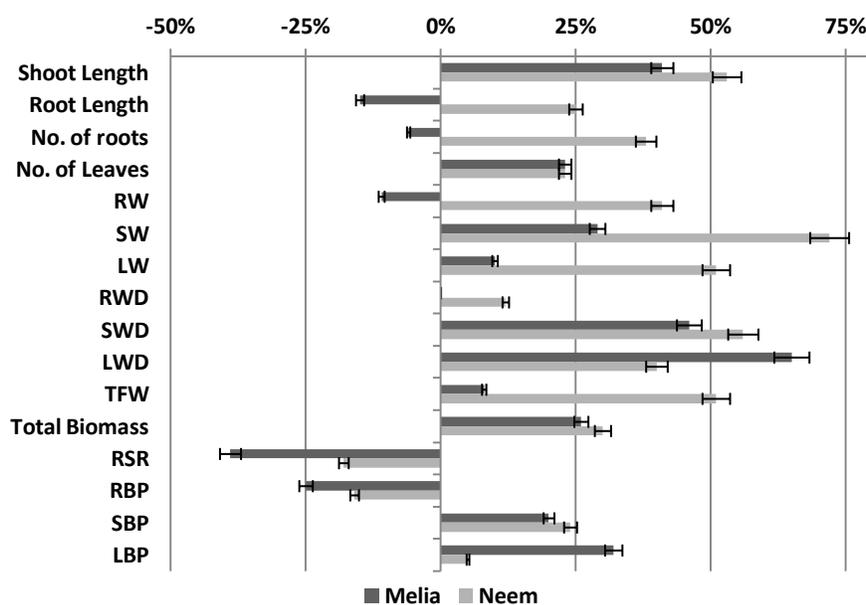


Fig. 2: The average increase (%) in growth under chamber, elevated (600 ppm, 900 ppm and 900 ppm with RH) relative to ambient (380 ppm) CO₂ for melia (dark shaded bars) and neem (light shaded bars). RW –Root Weight (Fresh); SW – Shoot Weight(Fresh); LW – Leaf Weight(Fresh); RWD –Root Weight (Dry); SWD– Shoot Weight(Dry); LWD – Leaf Weight(Dry); TFW- Total Fresh Weight; RSR – Root/Shoot Ratio; RBP - Root Biomass Partitioning ;SBP - Shoot Biomass Partitioning ; LBP - Leaf Biomass Partitioning.

Table 2: Short-term and long term responses of stomatal conductance (gs) and net CO₂ assimilation rate / photosynthetic rate (A) to elevated CO₂ in neem and Melia seedlings.

Species	Stomatal response		Photosynthetic response (NAR)	
	Short-term	Long-term	Short-term	Long-term
Neem	2.92*	2.30*	2.20*	1.63*
Melia	1.02	1.11*	0.93	1.21*

* indicates a ratio significantly different from 1.0, at P = 0.05.

Table 3: Simple Correlation between the overall biometric, physiological and biochemical parameters of neem and melia.

	AA	CHO	PTN	RS	DPPH	FLA	PHE	CA	SL	RL	RSR	TB	Pn	gs	Ci	E	A:B
AA	1																
CHO	.666**	1															
PTN	0.284	0.407	1														
RS	-0.036	-0.297	0.216	1													
DPPH	-.747**	-.882**	-0.092	0.384	1												
FLA	-.601**	-.691**	-0.146	.572**	.667**	1											
PHE	-.508*	-.552*	0.281	.475*	.628**	.804**	1										
CA	.543*	.694**	0.39	-0.391	-.532*	-.545*	-0.41	1									
SL	-0.057	-0.414	-.642**	.480*	0.172	0.33	-0.1	-.520*	1								
RL	-0.408	-.574**	-0.04	.530*	.567**	.583**	.517*	-.610**	0.441	1							
RSR	-0.365	-0.281	.491*	0.172	.468*	0.33	.607**	-0.203	-0.337	.690**	1						
TB	-0.224	0.013	-0.193	0.162	-0.009	0.33	-0.027	-0.043	0.352	0.278	-0.01	1					
Pn	-.619**	-.761**	-0.319	.492*	.724**	.598**	0.376	-.697**	.536*	.563**	0.166	0.316	1				
gs	-.525*	-.746**	-0.326	.666**	.703**	.603**	0.368	-.721**	.654**	.590**	0.09	0.226	.873**	1			
Ci	.488*	0.312	0.307	0.153	-0.175	-0.225	-0.151	0.296	-0.118	-0.119	-0.004	-0.25	-.469*	-0.292	1		
E	-.618**	-.822**	-0.303	.661**	.789**	.768**	.547*	-.740**	.611**	.660**	0.198	0.285	.881**	.959**	-0.261	1	
A:B	0.03	0.133	0.018	0.052	-0.217	0.289	0.186	-0.322	0.053	0.256	0.185	0.35	-0.11	-0.163	0.119	-0.028	1

AA - Free amino acids; CHO - Total Carbohydrates; PTN - Total Proteins; RS - Free Reducing Sugars; DPPH - DPPH antioxidant assay; FLA - Flavonoids; PHE - Total phenolics; CA - Carbonic Anhydrase activity; SL- Shoot Length; RL - Root Length; RSR - Root/Shoot Ratio, TB - Total Biomass; Net Photosynthetic Rate (Pn), Stomatal Conductance (gs), Intercellular CO₂ Concentration (Ci), Transpiration Rate (E), A:B - Chlorophyll a:b ratio.

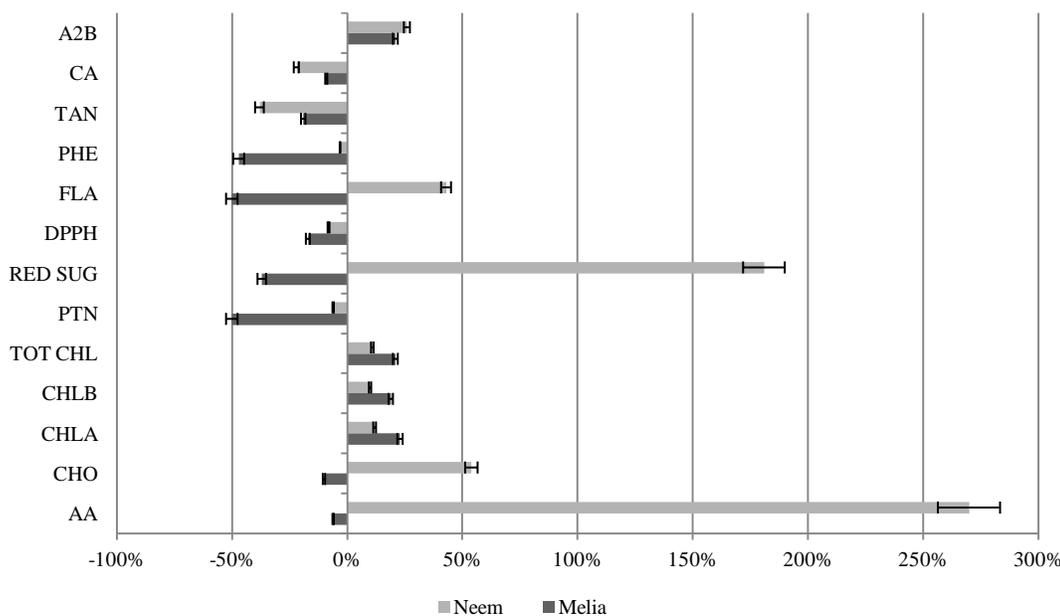


Fig 3: The average increase (%) in biochemicals under chamber, elevated (600 ppm, 900 ppm and 900 ppm with RH) relative to ambient (380 ppm) CO₂ for melia (dark shaded bars) and neem (light shaded bars). AA - Free amino acids; CHO - Total Carbohydrates; CHLA - Chlorophyll a; CHLB - Chlorophyll b; TOT CHL - Total Chlorophyll; PTN - Total Proteins; RED SUG - Free Reducing Sugars; DPPH - DPPH antioxidant assay; FLA - Flavonoids; PHE - Total phenolics; TAN - Tannins; CA - Carbonic Anhydrase activity; A2B - Chlorophyll a:b ratio.

3.2 Growth and Biochemical Measurements

Species level variations were significant for most parameters except RSR, LBP, and total biomass. Treatment effects showed significant differences for shoot length, total biomass, and partitioning in stem and leaves. Interaction effects of species and treatments were non-significant with respect to morphological/biometric traits (Table 1b).

Figure 2 presents the overall response of the two species to different treatments. All the parameters recorded higher values than control in neem. Except root dry weight, RSR, and RBP all other parameters recorded > 20 percent increase. Though RSR and RBD recorded a decrease with respect to control, it was less than 20 percent. In melia, all root related parameters showed a decrease in response to treatments. It varied from 0 to 40 percent (Fig. 2).

All biochemical parameters showed significant variations with respect to species, treatments and interaction effects at 1 per cent level (Table 1c). From Figure 3, it is very evident that treatments had a positive effect on the chlorophylls of both species including total and a:b ratio. CA, along with secondary metabolites, antioxidant activity, and proteins recorded a decrease with respect to the control in both species. However, with respect to amino acids, carbohydrates, flavanoids and reducing sugars, neem displayed an increase while in melia, it was lower than the control.

3.3 Correlation Studies

Simple correlations presented in Table 3 discuss the correlations between the biometric, physiological and biochemical parameters. Root length showed significant positive correlation ($P > 0.01$) with Pn, gs, E, reducing sugars, flavanoids, phenols, DPPH activity, and was negatively correlated with total carbohydrates. Similarly, shoot length showed significant positive correlation ($P > 0.01$) with Pn, gs, E, reducing sugars, and was negatively correlated with proteins and CA activity. Total biomass was not correlated with any of the parameters selected. Root Shoot Ratio showed significant positive correlation with proteins, DPPH activity, and phenols.

4. DISCUSSION

Elevated CO₂ stimulated changes in morphology and biomass allocation differently depending on species. Comparing the two species of Meliaceae, it was found that elevated CO₂ induced significant changes in plant morphology which includes the plant height, leaves and consequently increased shoot weight along with significantly enhanced biomass allocation toward stems and leaves. The increase in the percent of the total biomass in these two plants grown in the elevated CO₂ treatment (Fig 2) was consistent with a previous report of *Quercus cerruoides* and *Q. ilex* (27). It is postulated that elevated CO₂ atmosphere might show a preferential allocation to roots, thus increasing their root/shoot ratio [28]. However, in the present study, both species showed lower ratios than the ambient indicating a relatively higher

allocation of biomass to the shoots than the roots. The results suggest that both species had higher morphological plasticity for adjusting their biomass allocation to acclimatize to a new environment. Another feature observed was that between the two species, melia showed a preferential increase in shoot characteristics following treatments while the response of root related parameters was decreased. This could be attributed to the fast growing nature of the species.

Photosynthetic activity is a primary physiological process of biomass production. Any alteration in the photosynthesis process could lead to a change in total biomass accumulation. At elevated CO₂ concentrations, the average stimulation of total biomass in both species was high, which was in accordance with the observed photosynthesis stimulation.

Short-term experiments in tree species exposed to elevated CO₂ levels have shown increased photosynthetic rate up to 40-80% in pine seedlings (29). A 110% increased annual total net photosynthesis in elevated CO₂ than in an ambient CO₂ level [30]. Short-term exposure of C₃ plants to elevated CO₂ concentrations generally stimulates plant photosynthesis (31) and a proportional increase in biomass as a result of the improved competitiveness of CO₂ over O₂ as a substrate for the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (32). Compared to Melia, greater stimulation of photosynthesis by elevated CO₂ was observed in neem suggesting that this species might possess enhanced physiological regulation mechanisms at elevated CO₂ (Table 2). Increased stomatal conductance and photosynthetic responses under short and long term exposure of neem are indicative of the fact that neem has the ability to adapt to elevated CO₂ levels. Melia showed stability during short-term exposures while the long-term exposure observations warrants further and elaborate studies in the species with respect to gas exchange studies over longer periods of time to understand the acclimation mechanism in the species.

In general, photosynthetic activity is controlled by abilities of light energy transformation and utilization, CO₂ carboxylation in photosynthetic apparatus as well as stomatal conductance. There exists a clear difference in gas exchange between the two species under elevated CO₂ concentrations (Fig 1), which resulted in the different photosynthesis responses.

Experiments conducted in open-top chambers (OTCs) environment showed significant increases in light-saturated rates of photosynthesis in several C₃ plants grown at elevated CO₂ (33). Further, Ci/gs is a reflection of mesophyll efficiency, and this shows the significant inverse relationship with assimilation rate, Pn (34). The marked increase in net assimilation rates has been explained to be due to increased intercellular CO₂ concentrations (Ci) (35). Increase in stomatal conductance has been reported in a cultivar of *Avena sativa* [41] and the stomatal effect has been reported to depend on water supply in *Eucalyptus cladocalyx* [36]. In *Eucalyptus* 30 percent reduction in transpiration at 680 ppm of CO₂ and at saturating light intensity, compared to ambient conditions has been reported [37].

Plants respond to the changes in the environment not only by altering their physiological processes but also adjust biochemically. Studies on CO₂ enrichment report changes in biochemical composition, carbonic anhydrase, and nitrate reductase activity, and that the response is species specific [38]. Elevated CO₂ concentration of 600 ppm was favourable for enhancement of total chlorophylls in *A. indica* and *M. dubia*. This is contrary to reports that chlorophylls and stomatal conductance decreased in plants grown under elevated CO₂ compared to ambient CO₂ level [39]. Further, the accumulation of carbohydrates is reported to have a feedback inhibitory effect on photosynthetic rate, thereby reducing the chlorophyll content both a and b in this species [40]. However, this was not observed. The photosynthetic machinery was not severely affected due to high concentrations of CO₂ in both species, as is evident from Fig 3 which also favours the chlorophyll enhancement. Chlorophyll a/b ratio was observed to increase in both species following treatments. Variation in response of chlorophyll content to eCO₂ and elevated temperature has been reported in cultivars of *Avena sativa* some showing increase while others showing no response [41].

Foliar carbohydrates accumulate in the leaves of C3 plants grown at elevated CO₂ concentration and has been reported as one of the most pronounced and universal changes observed. Neem accumulated more carbohydrates and reducing sugars at elevated CO₂ concentrations. This is in accordance with reports [4, 42] that stimulation of carbon assimilation rates resulting from internal CO₂ concentrations in the leaves of plants in an enriched environment leads to increased stem and foliar biomass and higher levels of carbohydrates. Free reducing sugars and carbohydrates reduced in melia under the treatments, though they are generally reported to increase under eCO₂ [42 -46]. However, the notable increase in carbohydrate concentration could be due to non-reducing sugars which ultimately build up non-structural carbohydrates.

Neem and melia recorded a decline in the total protein concentration. This is in accordance with previous studies carried out under elevated CO₂ [47-50]. Plants lose the ability to take up soil nitrate (most common form of nitrogen) and convert it into organic compounds such as proteins at enriched CO₂ conditions. According to this hypothesis, it was reported that increasing carbon dioxide levels in the atmosphere could reduce crops' protein content by 20% [51]. Thus, elevated CO₂ has always found to have negative effects on foliar protein content. The free amino acid content of neem was found to increase with elevated CO₂. An adaptation response to elevated CO₂ stress was observed. This was in accordance with reports that the ratio of free minor amino acids at elevated CO₂ to that at current CO₂ was significantly greater [52]. On the contrary, melia recorded a significant decrease in total free amino acid contents when the high concentration of CO₂ was supplied. This may be due to the lack of sufficient nutrient intake particularly nitrogen assimilation [51].

Most amino acids get converted to their derivatives by free radicals. These free radicals were those that are produced from

phenols and polyphenols which when present in excess get converted from antioxidants to pro-oxidants [53]. Both species showed a notable decrease in phenolic and tannin contents. The decrease in phenolic content and carbohydrate accumulation reveals that carbon partitioning is favoured for carbohydrate accumulation rather than phenolic biosynthesis, especially in melia under elevated CO₂ levels. This was in accordance with the reports on other species [54]. It was previously reported that there was no significant difference in the antioxidant activity at elevated CO₂ [55] or enhanced flavonoid and antioxidant property [56]. Contrarily, the results of present study showed a notable decrease in the non-enzymatic antioxidant activity on CO₂ enrichment. Both the species recorded a uniform decline in antioxidant activity.

It is suggested that there is no direct correlation between high levels of starch accumulation and photosynthetic acclimation, and it is possible that increased starch levels, rather than feeding back to reduce photosynthetic capacity, enable the plants to avoid the down-regulation of photosynthesis, by preventing cycling and/or accumulation of soluble sugars [57]. A strong positive correlation between Pn and gs, with a large increase in Pn with a relatively small decrease in gs has been reported also in *Avena sativa* [41]. The increase of phenols under eCO₂ is consistent with the general response of plants found positively correlated with photosynthetic rates, increase in soluble sugars and total non-structural carbohydrates [58-59]. In the present study, shoot and root length showed significant positive correlation ($P > 0.01$) with Pn, gs, E and reducing sugars similar to the reports available. Root to shoot ratio was also significantly correlated with phenols.

In general, plants acclimate and adapt to the prevailing environmental conditions to optimize their growth and survival. Initially, when the atmospheric CO₂ concentration is increasing, the first effect is on the photosynthetic enzymes. All the other adjustments originate from this primary effect. The present study which attempted to understand effects of elevated CO₂ on two species of Meliaceae family revealed that growth responses, gas exchange parameters, and biochemicals varied significantly and was species specific. Growth response was better in the slow growing neem than in the fast growing Melia.

5. CONCLUSION

The study emphasizes the need to assess all important tropical tree species individually and not to generalize the response of tree species to elevated CO₂. Studies report the positive response of these three plantation species, namely teak, casuarinas and *Ailanthus* to increased CO₂ concentrations [60]. In addition, multiple environmental factors must be considered and their interactive effect on predicted climate change is needed to be studied and understood. The immediate and significant increase in photosynthesis can be exploited as a major strategic adaptation to mitigate the global rise in atmospheric CO₂. The study is indicative that neem adapts better than melia under changing environmental conditions, having higher short-term and long term responses, suggesting its relative success in the field under a future elevated CO₂ regime.

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