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## **Elevated CO<sub>2</sub> induced microclimatic flux on the growth and biochemical responses of *Arundo donax* L.**

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**Abstract**---Climate change is becoming a global environmental threat. Recent climate change report of the IPCC,2021 ascertains that Earth's average temperature will cross 1.5°C by 2040. Carbon dioxide ascend is the major reason for the accelerated levels of global temperature. Increasing levels of CO<sub>2</sub> in the atmosphere and their overwhelming effects on climate change warrants immediate strategies for CO<sub>2</sub> capture and storage. Carbon offset planting is an effective step in this direction. *Arundo donax* (Poaceae) is a C<sub>3</sub> grass with wider distribution and higher biomass production. It is considered as an excellent bioenergy crop. The present study has been undertaken to assess the CO<sub>2</sub> assimilation potential of *Arundo donax* grown under controlled experimental conditions, with elevated supply of CO<sub>2</sub>. Both morphological and biochemical changes were periodically monitored to assess their responses to elevated levels of CO<sub>2</sub>. The standardization studies and experimentation with plants was carried out for 15 days. The average day flux of CO<sub>2</sub> attributed by the plant in the treated chamber (-358.57±74.04 ppm) was significant, compared to control (-8.57±20.19ppm) and the results of standardization studies (-77.07±16.83). The respiratory increase of CO<sub>2</sub> was negligible. The percentage increase of carbohydrate concentration (24%) can be an index of the utilization of absorbed CO<sub>2</sub>, since its value was negative (-7.82) in control. Photosynthetic pigments decreased, but the values were not significant since reduction of pigment concentration is also noticed in plants under control. Culm diameter has increased

significantly under elevated CO<sub>2</sub>. Likewise, increase is also noticed in the number of tillers. Day flux value was attributed to the plant uptake. Percentage increase in carbohydrate concentration, consequent to fifteen days of treatment can be considered as an index of photosynthesis-mediated CO<sub>2</sub> uptake. Thus, *Arundo donax* can be employed as an ideal candidate for carbon offset planting.

**Keywords**--Biochemical responses, Day flux, Elevated CO<sub>2</sub>, Growth responses.

## 1. Introduction

Climate change is becoming a global environmental threat. Recent climate change report of the [1] ascertains that the Earth's average temperature will cross 1.5°C by 2040. Carbon dioxide ascend is stated to be the major reason for the accelerated levels of global temperature. Considering the present CO<sub>2</sub> levels and its overwhelming effects, there is an imperative need to develop an approach to capture and securely store CO<sub>2</sub>. Numerous studies were carried out on the capabilities of plants in the capture and store of CO<sub>2</sub> [2-6]. These studies evaluated the physiological and biochemical responses of selected plants to elevated CO<sub>2</sub>. Studies relating to the microclimatic changes associated with CO<sub>2</sub> enrichment and resultant responses of plants, especially grass species in regulating to increased CO<sub>2</sub> concentrations are scarce. In this perspective, the present study has been carried out to assess the responses of *Arundo donax* to increased flux of CO<sub>2</sub> and associated changes in micro climatic conditions.

*Arundo donax* is a C3 grass species of family Poaceae, distributed in temperate and hot regions all over the world. The adaptability of the species to grow in heterogeneous habitats with higher biomass makes them qualified as a biofuel /bioenergy crop [7]. The plant shows maximum biomass yields when growing in soils rich in water. The higher biomass production of *Arundo donax* distinguishes them from other herbaceous plants [7]. Higher biomass accumulation of *A. donax* is reported to be due to higher saturation level of the photosystem [8]. Also, it is reported that C3 grasses have higher CO<sub>2</sub> assimilation rates at enriched levels of CO<sub>2</sub> [9].

Considering the reported advantages of *Arundo donax* with regard to their photosynthetic ability and biomass production, an experiment has been carried out to assess their efficiencies in assimilating CO<sub>2</sub> and thereby offering services as ideal candidates in carbon offset planting. For this, acclimatized plants were grown in controlled growth chambers having CO<sub>2</sub> supply in specific dosages. The microclimatic conditions within the chambers were monitored, together with an estimation of the carbon flux and resulting impacts on the growth and biochemical responses of the plants.

## 2. Materials and Methods

### 2.1. Experimental design and monitoring of microclimatic conditions

Plantlets of *Arundo donax* were collected from pollution free natural habitats of Kannur district of Kerala, India. Plantlets of equal size were grown in poly bags of size 40x24x24 cm., each filled with soil, dried cow dung and sand in the ratio 3:1:1. They were then retained in a net house for acclimatization. After 6 months of growth, two sets of plants (3 each) were taken to the growth chambers having controlled CO<sub>2</sub> supply facility.

The controlled growth chambers of size 1.7mX1.7m X2.3m were made with PVC frames and polyethylene sheets of 1.0mm thickness (Fig I). The control chamber was provided with the facility for the supply of ambient air and the treated chamber with the supply of CO<sub>2</sub> – ambient air mixture at specific dosages, using a CO<sub>2</sub> cylinder, air compressor and a nebulizer. Both the chambers were equipped with the facility for the estimation of temperature and humidity (Billion bag digital wireless electronic Hygro-thermometer) and CO<sub>2</sub> using an automated CO<sub>2</sub> analyzer (NDIR type Infrared Gas Analyzer, Fuji Electric, Japan). An exhaust facility was also attached to each growth chamber to adjust the over dosages of CO<sub>2</sub>, if required. Semi-automated irrigation facility was also ensured within the chambers, during the period of experimentation.

Both the control and the treated chambers were sealed after introduction of the polybags containing *Arundo donax*. Daily, the control chamber was supplied with ambient air and the treated chamber with ambient air - CO<sub>2</sub> mixture for about 15 minutes. The concentration of CO<sub>2</sub> (ppm) in both the chambers were monitored in the morning and evening hours, together with the estimation of temperature and humidity. The experimentation was continued for 15 days. Day flux of CO<sub>2</sub> inside the chambers were calculated from the data of CO<sub>2</sub> levels in the morning and evening hours and night flux from the data of the evening hours with that of the next day morning data. The morphological and growth characteristics of plants inside the control and treated chamber were measured on the initial and final day of experimentation. Biochemical characteristics of the plants were measured at initial, 5<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> days of experimentation. A standardization study has also been undertaken for a period of 15 days to assess the day and night flux of carbon dioxide within the chambers in the absence of plants.

### 2.2. Determination of morphological and biochemical parameters

Morphological / growth attributes like plant height, tiller height, leaf length and breadth were assessed using a measuring scale. Screw gauge is used to measure culm diameter. Leaf number was counted manually and the leaf area was calculated from the values of leaf length, breadth and number.

Biochemical parameters were measured by homogenizing the leaf collected on the first and subsequent days of experimentation (5, 10 and 15<sup>th</sup> day). Standard procedures were followed for assessing pigments, total carbohydrates, total protein and total phenol content associated with the plants. Chlorophyll and

carotenoid contents were estimated using DMSO method [10]. Anthrone method [11] is employed for the estimation of total carbohydrates. Lowry's reagent and Folin ciocalteus phenol reagent was used for assessing the concentration of protein [12]. Protocol of [13] was used for the estimation of total phenol content.



Fig I Chambers (control and treated) installed for CO<sub>2</sub> enrichment experiments

### 3. Results and Discussion

#### 3.1 Microclimatic CO<sub>2</sub> flux - plant CO<sub>2</sub> uptake efficiency

Chamber experiments for measuring the CO<sub>2</sub> absorption capabilities of plants have been conducted previously by [14] and [15]. Similar experiments were conducted in the present study. However climatic conditions varied. Day time chamber temperature was between 30°C and 40°C. Humidity exceeded 99% in most of the occasions. Flux of CO<sub>2</sub>/microclimatic reduction in CO<sub>2</sub> concentration in treatment chamber with plants during the experiment showed higher values compared to control and standardization experiments (Fig II). Mean day flux data obtained from morning and evening microclimatic CO<sub>2</sub> concentrations in treatment chamber was  $-358.5 \pm 74.04$  ppm. The flux of CO<sub>2</sub> in standardization experiment (control) and in control chamber with plants was  $-30.71 \pm 17.94$  and  $-8.57 \pm 20.19$  ppm respectively. 50% reduction of CO<sub>2</sub> within 20 minutes during day time was noticed in previous experiment with *Ixora chinensis* [15]. Here a reduction of 67.23% was noticed in treatment chamber during day time by *Arundo donax*. Where there is only 7.57% decrease of microclimatic CO<sub>2</sub> concentration in standardization experiment (without plants) and provided with elevated CO<sub>2</sub>. This negligible reduction in standardization experiment (without plants) is assumed to be due to the assimilation by the chamber. Higher CO<sub>2</sub> gas exchange capacity of *Arundo donax* is previously established by [16] in open area. Also the present study establishes the higher CO<sub>2</sub> absorption potential of this plant in a microclimatic environment. Night flux values in CO<sub>2</sub> concentration inside treatment chamber are not comparable with control, except negligible respiratory increase between days 10 and 14 (Fig III).

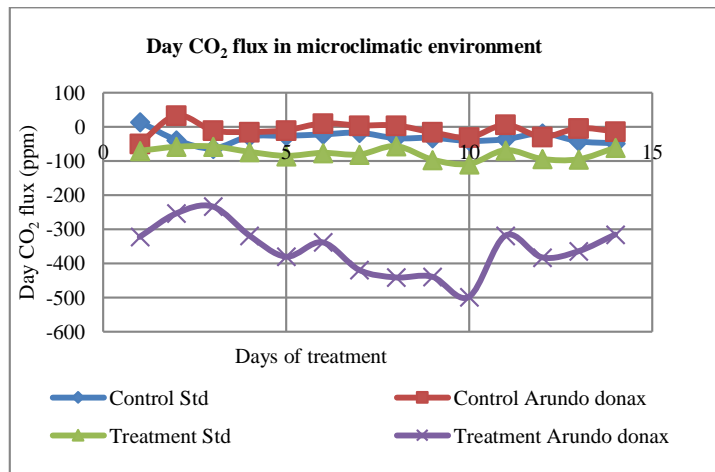


Fig II

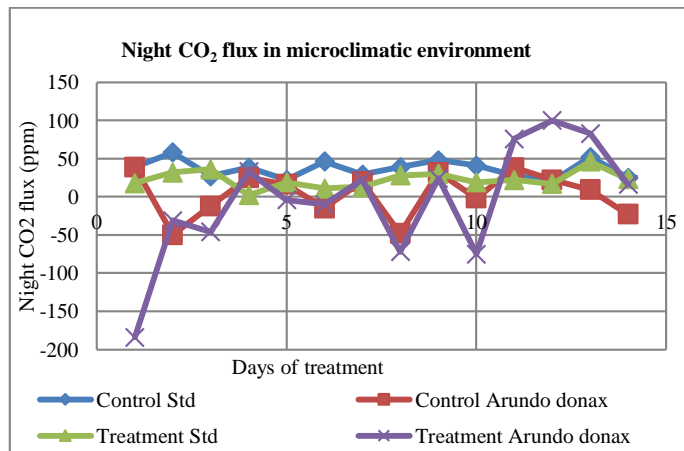


Fig III

### 3.2 Carbohydrate concentration and growth attributes – index of CO<sub>2</sub> uptake

Elevated carbon dioxide treatment on *Arundo donax* showed a decline in concentration of photosynthetic pigments (Table I). But the values were not significant since the reduction is also noticed in the control. However higher magnitude of reduction is visible in the treatment and it could be in connection with the percentage decrease in the number of leaves (Table III). Increased senescence prior to elevated CO<sub>2</sub> treatment is previously suggested by [17] and it may due to the least nitrogen allocation in older leaves as discussed by [18].

Table I. Changes in pigment concentration

	Days	Initial	15DAT*	% change
Control	Chl a	1.68±0.20	1.32±0.38	-21.429
	Ch b	0.48±0.10	0.34±0.10	-29.167
	Total Chl	2.16±0.27	1.66±0.48	-23.148
	Carotenoids	0.09±0.01	0.07±0.02	-22.222
Treatment	Chl a	1.91±0.38	1.27±0.29	-33.508
	Ch b	0.48±0.12	0.33±0.09	-31.250
	Total Chl	2.39±0.50	1.60±0.38	-33.054
	Carotenoids	0.11±0.02	0.07±0.01	-36.364

\*Days after treatment

Table II. Changes in the concentration of metabolites

Experimental condition	Days	Total Protein	Total carbohydrates	Total Phenol
Control	Initial	133.19±10.64	47.30±15.39	13.56±8.31
	5 DAT	113.94±21.40	45.40±5.44	23.29±11.77
	10 DAT	112.56±8.03	44.10±2.38	30.11±3.58
	15 DAT	138.13±11.69	43.60±3.29	39.96±2.67
% change		+3.71%	-7.82%	+194.69%
Treatment	Initial	138.23±2.88	37.5±4.83	22.07±8.19
	5 DAT	132.43±3.89	46.60±1.91	25.63±7.02
	10 DAT	125.86±9.03	42.80±2.69	33.97±1.88
	15 DAT	140.27±5.43	46.50±2.26	39.52±0.47
% change		+1.48%	+24%	+79.06%

Significant changes in chemical composition of plants prior to CO<sub>2</sub> treatment is previously discussed by [19]. In the present study, among the metabolites analyzed (Table II), total carbohydrate concentration was significantly increased in treatment (24%). Even though a significant decline in the number of leaves and decrease in pigment concentration, carbohydrate concentration may increase due to significant absorption of atmospheric CO<sub>2</sub>. Thus, it could be considered as an index of photosynthetic uptake of CO<sub>2</sub>. Previous studies on grass species support the present increase of carbohydrates [20,5]. Non-structural carbohydrate accumulation causes augmented growth and development in grasses [21]. The same author suggests increased CO<sub>2</sub> sequestration potential of grass species as a result of the storage of carbohydrate polymers.

Elevated CO<sub>2</sub> greatly influenced morphological characters of *Arundo donax*. Nevertheless significant positive influence is noticed only in culm diameter. Likewise there are reports on the augmentation in stem diameter of plants at elevated CO<sub>2</sub> [22]. Tillering is greatly influenced by carbon supply [23]. Contrary results were obtained in the present study since there is increase in tiller number and is not significant compared to control. Percentage change in leaf related parameters showed low values in treatment. Subsequent to this, leaf area of

plants showed greater decline. Increased senescence of leaves prior to CO<sub>2</sub> enrichment is the possible reason for leaf area reduction.

Table III. Percentage change in morphological characters

Morphological characters	% change in control chamber	% change in treatment chamber
Plant Height	+1.38	+1.04
No. Of tillers	+13.04	+10.34
No of leaves	-13.60	-27.84
Leaf length	+12.61	+5.95
Leaf breadth	+1.57	-44.72
Tiller height	+5.28	+3.45
Culm diameter	+1.82	+7.69
Leaf area	0	-57.89

### Conclusion

The results suggest that the C3 grass *Arundo donax* is responsive to higher atmospheric concentration of CO<sub>2</sub>. Initiation of these responses was from its potential CO<sub>2</sub> uptake capability. Treatment chamber exhibited greater day CO<sub>2</sub> flux or the higher reduction in microclimatic CO<sub>2</sub> concentration during the study period. Plants under treatment showed higher carbohydrate concentration also. It could be considered as an index of photosynthetic uptake of CO<sub>2</sub>. More experiments need to be undertaken to assess how *Arundo donax* respond to atmospheric CO<sub>2</sub> enrichment and how the plant behaves in open atmosphere with similar conditions. The results of this study are significant since the CO<sub>2</sub> flux data and subsequent changes in growth and biochemical characters will contribute to better understanding of the potential of *Arundo donax* as an ideal candidate for carbon offset planting.

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