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DISTINCTION IN MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN VALUABLE MEDICINAL HERB *ADHATODA VASICA* UNDER ELEVATED CO_2

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ABSTRACT

Increasing atmospheric CO_2 concentration is generally expected to enhance plant growth, allocation and chemical composition of alkaloids in medicinal plants. *Adhatoda vasica* is an important medicinal plant which is being cultivated commercially in India for different purposes. The response to the elevated CO_2 concentrations of various medicinal plants was studied with reference to growth and biochemical changes. It has gained a great interest from the pharmaceutical industry; the alkaloids vasicine from its sap has been shown to be an effective treatment for suppressant, cough and bronchodilator. It has a great importance in studying different parameters of *A. vasica.* The present study was carried out for growth and bio-chemical changes of *A. vasica* in different elevated CO_2 levels. Open top chambers (OTCs, diameter:3.0 m, height: 3.0 m) were used to expose plants to ambient and elevated CO_2 concentration (600ppm and 900 ppm). The experiment was conducted for a period five months. Carbon- di- oxide enrichment studies in special open top chambers help us in understanding the changes at individual Biochemical changes and plant growth. The bio-chemical analysis revealed that the highest alkaloid (609.07 mg/ml^{-1}) and flavonoid (242.37 mg/ml^{-1}) were recorded in control. Tannin (140.12mg/ml^{-1}) and saponin (45.96) were recorded in higher level at 900 ppm. In ambient condition, the highest phenol (207.66mg/ml^{-1}) was recorded. The plant growth revealed that the maximum fresh weight (15.76 cm), shoot length (37.25 cm) were observed in 900 ppm, and the maximum number of leaves (20) were observed in 600

ppm+RH. The maximum number of roots (15) and the longest root lengths (20.1 cm) were observed in 600 ppm. In the ambient condition, the above said characters were found to be in the lowest level.

KEYWORDS: *Adhatoda vasica*, Elevated CO_2, Bio chemical changes, Growth parameters.

INTRODUCTION

Increasing atmosphericlevels of CO_2 are expected to increase photosynthesis and the accumulation of carbohydrates over growth, maintenance and storage demands. Excess carbon based secondary metabolites, such as phenolic compounds and terpenoids and also to various storage carbohydrates (Bryant *et al*., 1983; Tuomi *et al*., 1988; Jones & Hartely., 2000). How the excess carbon allocated to secondary compounds seems to depend on the ontogenetic development of the plant (Reichardt *et al.*, 1984; Bryant& Julkunen-Titto, 1995; Jones & Hartely, 1999). Excess carbon also to be directed to synthesis of plantorgans or special tissues, such as cell walls and trichomes.

Plant chemistry might be strongly affected by the environmental changes, including elevated carbon dioxide (CO_2) concentrations, predicted under some climate scenarios (Bidart-Bouzat and Imeh-Nathaniel, 2008; Houghton *et al*., 2001). Atmospheric CO_2 concentrations have increased since the pre-industrial era and are predicted to double by the end of this century (Houghton *et al.*, 2001). Elevated CO_2 concentrations are expected to increase rates of photosynthesis, which in turn could enhance plant growth, biomass accumulation, plant size (Frenck *et al*., 2011; Klaiber et al. 2013c) and allocation to plant primary and secondary metabolites (Coley et al., 2002).

Medicinal plants are commonly used in treating orpreventing specific ailments or diseases and areconsidered to play a beneficial role in health care. Therefore, the study of plants as a resource of medicine hasbecome more important in the context of present globaltrade scenario where oxidative stress is found to be one ofthe major causes of health hazards. India is considered as atreasure house of valuable medicinal and aromatic plantspecies.

Adhatoda vasica, (Acanthaceae) commonly known as vasaka or malabar nut. The genus which is synonym to *Justicia*, has about 420 species. It is a small, evergreen, sub-herbaceous plant widely spread throughout the tropical region of south-east Asia and it is indigenous to India. It is one of the highly reputed plant species, utilized in indigenous systems of

medicines in India for over 2000 years. The plant is valued for containing bronchodilator alkaloids, mainly vasicine. All parts of the plant are used in herbal medicine and particularly the leaves are credited with insecticidal and parasiticidal properties. The root is useful in strangury, leucorrhoea, bronchitis, asthma, bilious vomiting, sore eyes, fever and gonorrhoea. It is a valuable antiseptic, antiperiodic and anthelmintic.

MATERIALS AND METHODES

Enrichment of CO_2.

The present study was conducted at the Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu. The selected medicinal plants were grown Inside the open top chambers (OTCs) of 3 m diameter and 10 m height lined with transparent PVC sheets (0.125 mm thickness) with a CO_2 levels of 600 mol mol^{-1}. Pure CO_2 gas was used for the enrichment. Similarly OTCs were maintained at elevated temperatures (Ambient +4ºC) under ambientCO_2(380 mol mol^{-1}). Controls were maintained in open field outside OTCs, with ambientCO_2(380 mol mol^{-1}). CO_2 was provided throughout the day and night (24 h period). The experiments were laid in a Complete Randomized Design. The period of CO_2 enrichment was 180 days. A software facility called Supervisory Control and Data Acquisition (SCADA) was used to continuously control record and display the actual and desired CO_2level, relative humidity and temperature in each OTC by feedback control loop passing through Programmable Logical Controllers (PLC) (Buvaneswaran*et al.*, 2010). The set that was maintained in the open served as the control under ambient conditions while the set maintained inside the chamber under ambient CO_2conditions was used to eliminate the effects of the chamber on the response of the clones.

Bio-chemical analysis.

The samples were air dried for about one week and ground into fine powder.150 mg of each of the powder were weighed separately and dissolved in 3.0ml of methanol and water. For the water extracts, the solution was heated to 100°C and maintained this temperature for 15Minutes. They were covered, mixed and kept for 8 hours with intermittent shaking for every 30 minutes and then allowed to stand for 48 hours for extraction. The solutions were subsequently shaken and filtered using Whatman filter paper. The filtrates were allowed to evaporate to dryness. The residue was dissolved in 5ml of 90% methanol and water for the organic and aqueous solvent samples. These were stored at 15°C and then used for biochemical screening using the standard procedure described by Trease and Evans (1983)

and Kokate (1994). The presence of alkaloids and flavonoids were further confirmed by Thin Layer Chromatography (TLC). Protein and carbohydrate was determined by Lowry's method and Anthrone method respectively. The secondary metabolites such as phenol, tannic acid and flavonoids were quantitatively determined by Folin-Ciocalteau reagent method, Folin-Denis method and Aluminium chloride colorimetric method respectively. Carbonic anhydrase was estimated by Wilbur and Anderson method (1948) and Chlorophyll by Arnon method (1949).

Statistical Analysis

The data were subjected to analysis of variance for completely randomized design with five replications. A full-factorial multivariate general linear model (GLM) analysis was conducted using SPSS to determine whether there was significant variation in the different gas exchange and biochemical characteristics between different CO_2 conditions within the plants. Post hoc range tests using Waller Duncan t-test was performed to group the significantly different plants.

RESULT AND DISCUSSION

The bio-chemical analysis revealed that the highest alkaloid (609.07 mg/ml^{-1}) and flavonoid(242.37 mg/ml^{-1}) were recorded in control. Tannin(140.12mg/ml^{-1}) and saponin(45.96) were recorded in higher level at 900 ppm. In ambient condition, the highest phenol(207.66mg/ml^{-1}) was recorded. The plant growth revealed that the maximum fresh weight(15.76 *cm*), shoot length(37.25 *cm*) were observed in 900 ppm, and the maximum number of leaves(20) were observed in 600 ppm+RH. The maximum number of roots (15) and the longest root lengths (20.1 *cm*) were observed in 600 ppm. In the ambient condition, the above said characters were found to be in the lowest level.

Effect of elevated CO_2 in Alkaloid

The highest alkaloid was observed under control condition (609.07 mg/ml^{-1}). The similar results revealed that the total alkaloid accumulation in shoot of *C.roseus* was found increased significantly under droughtstress. The content of alkaloids in *C. roseus* has beenfound influenced by individual factor, such as stage ofplant growth, drought and other stress (Misra and Gupta, 2006; Osman, *et al*., 2007). The leaves and stem are thesources of the natural dimeric alkaloids vinblastine and vincristine that are essential parts of most anti-cancer chemotherapies (Heijden, Jacobs Denise, Snoeijer, Hallard, and Verpoorte, 2004).

This situation changed when *Stuhlfauth et al.* (1987) studied the individual and combined effects of atmospheric CO_2 enrichment and water stress on the production of secondary metabolites in the woolly foxglove (*Digitalis lanata* EHRH), which produces the cardiac glycoside *digoxin* that is used in the treatment of cardiac insufficiency. Under controlled well-watered conditions, a near-tripling of the air's CO_2 content increased plant dry weight production in this medicinal plant by 63%, while under water-stressed conditions the CO_2-induced dry weight increase was 83%. In addition, the concentration of digoxin within the plant dry mass was enhanced by 11% under well-watered conditions and by 14% under conditions of water stress.

Effect of elevated CO_2 in Flavonoid

These efforts enabled Moghaddam *et al* (2011) report that the daily two-hour 400-ppm increase in the controlled environment chambers' atmospheric CO_2 concentration led to a 193% increase in *C. asiatica* leaf biomass, a 264% increase in plant water use efficiency, as well as a 171% increase in leaf total flavonoid content, which findings led the six scientists to conclude that "collectively, the enhancement in yield and quality provides an economic motivation to produce a consistent pharmaceutical-grade product for commercial purposes," via what they described as "controlled environment plant production." And it also stands to reason that the on-going rise in the atmosphere's CO_2 concentration should be gradually increasing the medicinal potency of *C. asiatica* plants either growing wild or cultivated out-of-doors.

Effect of elevated CO_2 in Tannin

A substantially different result was obtained in an earlier study of aspen leaves that was conducted by McDonald et al. (1999), who grew aspen seedlings in controlled environment greenhouses that were maintained at either ambient (387 ppm) or elevated (696 ppm) CO_2 concentrations under conditions of either low or high light availability (half and full sunlight, respectively) for 31 days after the mean date of bud break. In this case it was determined that under low light conditions, the CO_2-enriched seedlings exhibited an increase of approximately 15% in leaf condensed tannin concentration, while under high light conditions the CO2-induced increase in leaf condensed tannin concentration was a whopping 175%.

Effect of elevated CO_2 in Phenol

In a five years experiment with *Populus nigra* L., elevated CO_2 and nitrogen fertilization, alone or incombination, did not affect lignin concentrations in wood. Soluble phenolics and

soluble proteins in wooddecreased slightly in response to elevated CO_2. Higher nitrogen supply stimulated formation of carbon basesecondary compounds and increased protein concentrations (Luo *et al*., 2008).

Effect of elevated CO_2 in Saponin

In this study we examined effects of CO_2 enrichmentand herbivore damage on interactions between alfalfa (Medicagosativa L.), cotton (GossypiumhirsutumL.), The herbivore-induced response of alfalfa was only recently discovered andincludes increased foliar levels of saponins and flavonoids (Agrell *et al.* 2003). *S. littoralis* is ageneralist defoliator and a major pest on both alfalfaand cotton (Brown and Dewhurst 1975).

Currently, an increasing attention is paid to theconsequences of global changes, such as elevated CO_2, O_3, UV-light and temperature, on inducibility of plant defence compounds as well as on their directand indirect impact on plant interactions with herbivoresand pathogens in terms of environmentalbiodiversity and shaping future ecosystems (Agrell et al. 2004; Bidart-Bouzat and Iimeh-Nathaniel 2008; Pearson et al. 2008; Iriti and Faoro, 2009). Abiotic and biotic stresses cause fluxes between plantprimary and secondary metabolism resulting in adiversion of available resources from growth todefense, what can result in excessive production of secondary metabolites including saponins. It may besometimes detrimental for the plant in terms of allocation costs and autotoxicity, though such enrichment in bioactive compounds may be favourable forplant resistance against pests and pathogens, as wellas for adaptation to changing environment andtolerance towards worsening climatic conditions. Moreover, increased level of plant bioactive compoundsmay alter competitive balance between plantspecies and change density of populations of importantherbivores and involved predators, as it wasdemonstrated for growing content of triterpene saponins in alfalfa influencing trophic cascades of arthropods (Agrell *et al.*, 2006; Pearson *et al.*, 2008).

Effect of elevated CO_2 on growth

A 400–700 ppm (75%) increase in atmospheric CO_2 concentration led to a 48% increase in abovegroundgrowth and a 56% increase in belowground (bulb) growth of *Hymenocallis littoralis*. These responses are similar tothose observed in other crop plants in terms of total productivityenhancement and differences in above- and belowgroundgrowth stimulation. For root crops such ascarrot and radish, for example, the biomass of the primaryroot storage organ is typically enhanced slightlymore than the aboveground biomass (Idso, Kimball andMauney, 1988). In contrast, plants such as cotton andsoybean, which do not possess root

storage organs, generallyexperience a more equal above- and belowgroundgrowth stimulation (Idso, Kimball, and Mauney, 1988), as we have also observed in sour orange trees (Idso andKimball, 1991).

More recently, Teixeira Da Silva *et al*. (2006) grew ornamental *Spathiphyllum* cv. Merry plantlets for a period of five weeks in novel culture vessels on a sugar-free liquid medium at low light intensity in controlled-environment chambers maintained at atmospheric CO_2 concentrations of either 375, 1000, 2000 and 3000 ppm. Relative to the growth experienced by the plantlets exposed to ambient air of 375 ppm CO_2, the plantlets exposed to 1000, 2000 and 3000 ppm CO_2 produced 39%, 81% and 129% more *shoot* dry weight, respectively, plus 316%, 639% and 813% more *root* dry weight, respectively, for corresponding *total* CO_2-induced biomass enhancements of 61%, 127% and 185

An increase in leaf mass per unit area is common responseto CO_2 enrichment (Mousseau and Saugier 1992). However, it is unclear whether this response is mainly attributable to accumulationsof starch or other nonstructural carbohydrates (Delucia et al. 1985, Wong 1990), or to an increase in leafthickness caused by an increase in the number of palisade celllayers (Thomas and Harvey 1983). In any case, the increase in LMA led to a slight decrease in nitrogen concentration per unitdry mass, which was mostly attributable to a dilution effect, because nitrogen content per unit area either increased orremained unchanged in response to elevated CO_2.

The maximum number of Leaves was observed in 600 ppm+rh (20Nos.), followed by 600 (110Nos.). The similar results proposed by Rogers *et al.*, (1983); Sionit *et al*., (1981) and Cure *et al.*, (1989), higher leaf area production has been reported in many similar studies due to increase in number of leaves, branches increased tillering and due to increase in leaf expansion rates.

Effect of elevated CO_2on root

In reviewing the scientific literature pertaining to atmospheric CO_2 enrichment effects on belowground plant growth and development, Weihong *et al*. (2000) briefly summarize what is known about this subject. They report that atmospheric CO_2 enrichment typically enhances the growth rates of roots, especially those of fine roots and that CO_2-induced increases in root production eventually lead to increased carbon inputs to soils, due to enhanced root turnover and exudation of various organic carbon compounds, which can potentially lead to greater

soil carbon sequestration. In addition, they note that increased soil carbon inputs stimulate the growth and activities of soil microorganisms that utilize plant-derived carbon as their primary energy source; and they report that subsequently enhanced activities of fungal and bacterial plant symbionts often lead to increased plant nutrient acquisition.

In a much more narrowly-focused study, Crookshanks*et al*. (1998) sprouted seeds of the small and fast-growing *Arabidopsis thaliana* plant on agar medium in Petri dishes and grew the resulting immature plants in controlled environment chambers maintained at atmospheric CO_2 concentrations of either 355 or 700 ppm. Visual assessments of root growth were made after emergence of the roots from the seeds, while microscopic investigations of root cell properties were also conducted. In pursuing this protocol, the scientists learned that the CO_2-enriched plants directed a greater proportion of their newly-produced biomass into root, as opposed to shoot, growth. In addition, the young plants produced longer primary roots and more and longer lateral roots. These effects were found to be related to the CO_2-induced stimulation of mitotic activity, accelerated cortical cell expansion, and increased cell wall plasticity.

Effect of elevated CO_2 on growth of Adhatoda vasica.

parameters	**ambient**			**CHAMBER 1 (600ppm)**			**CHAMBER 2 (control)**		
month	1	3	5	1	3	5	1	3	5
Plant Fresh weight g)	11.23	11.76	11.76	8.925	8.54	8.54	10.11	12.54	13
Plant height(cm)	35.25	40.5	40.5	41.75	50	50	40.1	38.5	35.5
Shoot length (cm)	19.75	24	4.25	24.5	31	26.25	22.5	27	23
shoot fresh weight(g)	9.945	8.18	8.18	7.835	15.53	12.06	8.92	9.47	1.22
shoot dry weight(g)	4.6	4.75	4.75	2.765	12.33	2.75	3.66	7.15	0.46
Root length(cm)	15.5	16.5	16.5	17.25	19	19	17.6	11.5	12.5
Root fresh weight(g)	1.29	3.57	3.58	1.09	3.01	3.05	1.20	2.27	0.33
root dry weight(g)	0.58	1.6	1.65	0.46	2.65	2.28	0.6	1.05	0.18
No of leaves	13.0	11	12	13.0	22	29	13.0	15	12
No of primary root	15	13	13	13.5	15	36	11	14.5	4
No of secondary root	192.5	76	76	110.0	114	300	142	167	49

parameters	**CHAMBER 5 (900ppm)**			**CHAMBER 6 (600ppm +RH)**		
month	1	3	5	1	3	5
Plant Fresh weight g)	16.74	15.16	17	12.17	25.23	25.24
Plant height(cm)	43.75	44	46	42.95	45.5	50
Shoot length (cm)	25.75	28	13	24.95	29	34.25
shoot fresh weight(g)	13.04	11.75	1.59	8.80	18.58	18.60
shoot dry weight(g)	5.195	9.85	0.46	3.17	12.05	12.06
Root length(cm)	18	16	9	18	16.5	16.3
Root fresh weight(g)	3.70	3.41	0.47	3.37	6.65	5.6

root dry weight(g)	1.04	2.1	0.7	0.78	4.6	1.25
No of leaves	24	30	25	19	20	14
No of primary root	14.5	17	14	14	19	14
No of secondary root	258	220	200	297	305	125

Effect of elevated co2 on bio chemical of adhatoda vasica

Treatments	**Alkaloid**			**Flavonoid**			**Phenol**		
Months	1	3	5	1	3	5	1	3	5
Ambient	352.6	358.6	250.4	102.6	114.8	143.8	195.6	197.8	207.66
Control	452.6	462.3	609.7	224.9	231.5	242.37	102.9	108.4	111.6
600ppm	459.4	475.6	468.4	159.6	167.8	175.3	147.3	151.6	147.3
600ppm+RH	367.5	395.6	400.5	168.4	173.6	187.6	122.3	128.4	131.6
900ppm	489.3	476.7	488.5	168.2	175.9	171.3	156.8	169.4	144.8

Treatments	**Tannin**			**Saponin**		
Months	1	3	5	1	3	5
Ambient	98.5	95.4	94.6	22.3	24.6	27.1
Control	87.6	88.3	82.3	23.2	25.7	26.6
600ppm	94.6	96.7	99.8	33.5	34.9	36.1
600ppm+RH	88.3	87.1	88.5	33.8	36.3	37.5
900ppm	132.8	136.5	140.12	40.2	42.5	45.96

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