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Original Research Article

Effect of Elevated Carbon dioxide (CO_2) on Silver Cock's Comb (*Celocia agentea)* Vegetable's Growth Parameters and Antioxidant Properties

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ABSTRACT

Rising atmospheric carbon dioxide (CO_2) levels significantly impact plant functions, including growth and nutritional quality. Despite the known benefits of elevated carbon dioxide (CO_2) on crop yield, limited research investigates its effects on the nutritional content of tropical leafy vegetables like *Celosia argentea*. This study examines how increased CO_2 affects growth parameters as well as antioxidant properties in *Celosia argentea*. Results demonstrate that increasing carbon dioxide (CO_2) exposure positively influences plant growth, with higher leaf counts and enhanced growth observed at elevated carbon dioxide (CO_2) concentrations. Proximate analysis reveals changes in moisture, ash, fat, fiber, carbohydrate, and protein content, suggesting complex responses to elevated carbon dioxide (CO_2). Notably, antioxidant activity varies across carbon dioxide (CO_2) levels, with different assays showing contrasting trends. Lipid Peroxidation levels fluctuate, while reduced glutathione levels rise with increasing carbon dioxide (CO_2), indicating a potential stimulation of antioxidant production. Furthermore, inhibitory activities against alpha-amylase and alpha-glucosidase increase with carbon dioxide (CO_2) levels, suggesting potential health benefits in managing conditions like diabetes. Overall, this research underscores the importance of considering carbon dioxide (CO_2) levels in assessing the nutritional and medicinal value of tropical leafy vegetables like *Celosia argentea*.

Keywords: Atmospheric Carbon Dioxide (CO_2); *Celosia argentea*; Growth Parameters; Antioxidant Properties

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INTRODUCTION

Increasing global levels of carbon dioxide (CO_2) represent a significant factor affecting not just the environment but also numerous plant functions, encompassing growth, productivity, and nutritional quality (Giri *et al.,* 2016). Increased aerial carbon dioxide has been shown to cause increased yield of crops including vegetables such as lettuce and carrot (Makino & Mae, 1999; Dong *et al.,* 2018). The chemical energy formed during photosynthesis is sugars generated from water and carbon dioxide which is trapped from the atmosphere (Bassham & Lambers, 2024). Photosynthesis is very essential for plant growth and it requires carbon for its stimulation (Makino & Mae, 1999). An elevated level of carbon dioxide (CO_2) in the atmosphere supports photosynthesis,

thereby enhancing plant growth (Boretti & Florentine, 2019). One of the tropical leafy vegetable crops, *Celosia argentea* plant which is a member of Amaranthaceae family is commonly used for treating of jaundice, gonohorrea, diabetes, wound healing, and folklore practice (Syed *et al.,* 2018). *Celosia argentea* is a very important leafy and tasty vegetable crop with high nutritional value among all other species (Aladesanwa *et al.,* 2001). It contains compounds such as diterpenes, flavonoids, steroids, phenolics and in the seeds is fixed oil (Wu *et al.,* 2011). These different compounds that it contains contribute to its various physiological effects (Thorat, 2018). Despite the nutritional content of *Celosia argentea*, no information is provided regarding the comprehensive effect of increased carbon dioxide on its nutritional content (Moretti *et al.,* 2010). Thus, the need to assess the effect of elevated carbon dioxide on the antioxidant potency and growth parameters of *Celosia argentea*.

MATERIALS AND METHODS

Chemicals

Distilled water which was produced using glass distillation unit and all of the chemicals as well as the reagents used in the analysis were of laboratory quality.

Sample Preparation

Celosia argentea was cultivated in carefully regulated atmospheric conditions. The level of carbon dioxide ($CO_{2)}$ in chambers 1, 2, 3, and 4 were 0.4, 0.6, 0.8, and 1.0mg/g all through the period of the experiment, respectively, on *Celosia argentea*. The control group was not exposed to carbon dioxide ($CO_{2)}$. For seven days until the experiment was concluded, the leaves number and the plant growth effectiveness were numerated. The samples were collected on the last day of the experiment, dried, and weighed (5g in 20mL of distilled water). The mixtures were violently shaken with an orbit shaker then after being chilled, the filtrate was utilized for subsequent enzymes as well as antioxidants analysis.

Phytochemical Analysis

Mineral and Nutrient Analysis

The AOAC (1975) approach was used to determine the plant's crude fiber, fat, ash, and carbohydrate content using *Celosia argentea* as the target sample. The protein content was calculated using micro-Kjeldahl method and flame atomic absorption spectrophotometer[13] and a flame photometer was used to measure the mineral content (Zn, Ca, Fe, and Mg), Na, and K respectively (Baur & Ensminger, 1977).

Total Phenol Determination

The total phenol content of extracts from leaves of *Celosia argentea* were assessed using a combination of reactions made from suitably 7.5% sodium carbonate, 10% Folin, and diluted aqueous extracts. The calculation of the total amount of phenol was based on gallic acid equivalent (GAE), determined by monitoring of absorbance at 765nm over 40 minutes incubation period at 45°C (Williams, 1978; Singleton *et al.,* 1999).

Total Flavonoid Determination

Using a method involving appropriately leaf samples, 10% $AlCl_{3,}$ methanol, and 1M potassium acetate, the overall concentration of flavonoids in extracts from *Celosia argentea* leaves were evaluated. Following 30 minutes incubation at 25°C, absorbance was recorded at 415nm, and the content of all flavonoids was determined as the equivalent of quercetin (QUE) (Meda *et al.,* 2005).

Antioxidant Analysis

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Scavenging Ability Determination

The capacity of *Celosia argentea* leaf extracts to scavenge the 2,2-azinobis-3-ethylbenzo-thiazoline-6-sulfonate (ABTS) radical was assessed in a dark reaction using ABTS solution and appropriately water-diluted extracts. Following 15 minutes incubation period, absorbance was recorded at 734nm, then trolox equivalent antioxidant capacity (TEAC) was used to measure the ABTS scavenging ability (Re *et al.,* 1999).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Determination

The ability of extracts from *Celosia argentea* leaves to neutralize 1, 1-diphenyl–2-picrylhydrazyl (DPPH) radicals was examined using appropriately combined water-diluted extract and DPPH. This blend underwent 30-minutes incubation in darkness, followed by absorbance measurement at 516nm. The DPPH scavenging efficiency was then calculated as a percentage of inhibition (Gyamfi *et al.,* 1999).

Ferric-reducing Antioxidant Property (FRAP) Determination

Assessment of the reducing capability of *Celosia argentea* leaf sample extracts was conducted within reacting mixtures containing suitably diluted samples, phosphate buffer (0.2M, pH 6.6), and 1% potassium

ferricyanide. This interaction proceeded at temperature, 50°C for a duration of 20 minutes. Subsequently, 10% of trichloroacetic acid was introduced, followed by centrifugation at 3,000g for ten minutes. Next, 1mL of the residue was combined having a comparable quantity of distilled water and 200µL of 0.1% ferric chloride. After measuring absorbance at 700nm, the ferric reducing antioxidant power (FRAP) was then calculated equivalent (AAE) (Oyaizu, 1986).

Hydroxyl Ion (OH) Scavenging Determination (Fenton reaction)

The *Celosia argentea* leaf extract's ability to mitigate hydroxyl radicals generated from the degradation of deoxyribose induced by Fe^{2+} and H_2O_2 was examined. This assessment was carried out within a reaction mixture consisting of water-diluted extracts, 20 mM deoxyribose, phosphate buffer (0.1 M, pH 7.0), 500µM $FeSO_4$, and distilled water. The process of incubation occurred at 37°C for thirty minutes, followed by the addition of 28% trichloroacetic acid (TCA) and 0.6% thiobarbituric acid (TBA). The mixture was then incubated at 100°C for 20 minutes, and absorbance was measured at 532nm. The hydroxyl radical scavenging ability was established as the percentage of inhibition (Halliwell & Gutteridge, 1981).

Nitric Oxide (NO) Scavenging Determination

Celosia argentea leaf extracts efficacy in scavenging nitric oxide (NO) radicals were assessed within an interaction mixture comprising appropriate ratio of water-diluted extracts and 25mM sodium nitroprusside. The mixture was then undergone incubation for two hours at 37°C. Subsequently, ther was introduction of Griess reagent, and at 570 nm, absorbance was measured. The nitric oxide scavenging capability was computed as the percentage of inhibition (Marcocci *et al.,* 1994).

Iron (Fe^{2+}) Chelating Determination

Evaluation of Fe^{2+} chelation by *Celosia argentea* leaf extracts was conducted within a solution comprising recently prepared 0.5mM $FeSO_4$, Tris–HCl (0.1 M, pH 7.4), 10% saline, as well as extracts in appropriate proportions. At 37°C, incubation took place for 5 minutes followed by the addition of 0.25% 1,10-phenanthroline after which the absorbance was taken at 510nm, and the Fe^{2+} chelating efficacy was detected as the percentage of a suppression (inhibition) (Minotti & Aust, 1987).

Lipid Peroxidation (LPO) Assay

The evaluation aimed to assess the decrease in malondialdehyde (MDA) as a result of Fe2+-triggered lipid peroxidation (LPO) by *Celosia argentea* leaf extracts. This assessment was conducted within a combination of reactions comprising homogenate of tissue, Tris–HCl buffer (100 mM, pH 7.4), appropriate amounts of the extract, and 0.25mM of freshly produced $FeSO_4$. The solution was then heated at 37°C for one hour. Subsequently, 8.1% sodium dodecyl sulfate (SDS), acetic acid/HCl buffer (pH 3.4), and 0.8% of TBA were added, and then furthered heated at 100°C for one hour. Measurement of thiobarbituric acid reactive substances (TBARS) at 532nm was conducted. The degree of lipid peroxidation was quantified as the proportion of MDA produced in contrast with the control group (Ohkawa *et al.,* 1979).

Enzyme Assays

α- Amylase Inhibition

The suppressive impact of *Celosia argentea* leaf extracts on α-amylase were determined using pancreatic α-amylase (EC 3.2.1.1) solution. The assay involved mixing 1% starch solution (500µL) with buffer solution and incubating the mixture for ten minutes at 25°C. The interaction was terminated with the addition of 1.0mL of DNSA solution, and next by heating at 100°C for five minutes. Once cooled, the absorbance was measured at 540 nm after the addition pf 10Ml of distilled water (H_2O). The α-amylase inhibitory action was then evaluated (Ademiluyi & Oboh, 2013).

α-Glucosidase Inhibition

A 50µL fraction as well as 100µL of α-glucosidase solution were combined and heated for 10 minutes at temperature, 25°C prior to the inclusion of p-nitrophenyl-α-D-glucopyranoside solutions at a concentration of 5 mmol/l. The resulting solution was then heated for five minutes at 25°C. Readings (absorbance) was taken at 405 nm to assess α-glucosidase inhibition by *Celosia argentea* (Apostolidis et al., 2007).

Angiotensin-Converting Enzyme (ACE) Inhibition

The ACE inhibition assay was conducted by employing a somewhat revised technique. Phenolic extracts were diluted (0–50 µL), and 50 µL of ACE (EC 3.4.15.1) solution (4mU/mL) was streamed at 37°C for 15 minutes. The reaction of enzymes commenced upon the addition of 150µL of 8.33mM Bz–Gly–His–Leu substrate together with 125mM Tris-HCl buffer (pH 8.3) to the solution. After the period of heating which elapsed for 30 minutes at 37°C, the interaction got terminated by the addition 250 µL of 1 M HCl. The bond between Gly–His cleaved, then Bz–Gly which was formed by the reaction got extracted using 1.5 mL ethyl acetate. Subsequently, the solution was separated using a centrifuge in order to obtain the ethyl

acetate layer; after being moved to a sterile test tube, 1 mL of the ethyl acetate layer was evaporated. The sediment was dissolved in distilled water, and 228 nm was used to assess its absorption. Percentage inhibition was used to express the ACE inhibitory actions.

RESULTS

Leaf Count and Growth Performance of *Celosia argentea*

Table 1 shows variation in leaf count across different carbon dioxide (CO_2) levels over the course of the experiment. Initially, on Day 1, substantial variability in leaf count was observed, with the highest counts recorded at 0.8mg/g and 1.0mg/g CO_2. This trend persists through Day 7, where the highest leaf count consistently appears at 1.0mg/g CO_2, followed by 0.8mg/g CO_2. As the experiment progresses, subsequent days show intensified differences in leaf count, with a consistent superiority of 1.0mg/g CO_2. Table 2 shows the impact of varying carbon dioxide (CO_2) levels on plant growth over time. Initially, on Day 1, higher CO_2 concentrations, particularly at 0.8mg/g and 1.0mg/g, led to a noticeable increase in plant growth. This stimulating effect persisted through subsequent days, with growth enhancement remaining evident up to Day 7, consistently strongest at 1.0mg/g CO_2. As the plants matured, this trend intensified, with Days 14 and 21 demonstrating significant differences in growth performance, consistently favoring higher CO_2 concentrations.

Table 1: Number of Leaves of *Celosia Argentea* Grown at Different Level of CO_2

Days	Control	0.4mg/g	0.6mg/g	0.8mg/g	1.0mg/g
1	8.95 ± 0.07^{c}	7.95 ± 0.07^{d}	9.00 ± 0.01^{b}	11.10 ± 0.01^{a}	7.95 ± 0.01^{d}
7	12.05 ± 0.07^{b}	12.12 ± 0.10^{b}	9.95 ± 0.07^{c}	14.02 ± 0.01^{a}	12.11 ± 0.10^{b}
14	26.75 ± 0.43^{a}	9.85 ± 0.20^{d}	14.02 ± 0.01^{c}	17.81 ± 0.30^{b}	14.75 ± 0.40^{c}
21	27.12 ± 0.01^{a}	18.05 ± 0.07^{c}	16.95 ± 0.07^{d}	22.15 ± 0.22^{b}	18.22 ± 0.31^{c}

The mean ± standard deviation is represented by the values. Significant ($p<0.05$) values are indicated by a different superscript letter in the same column

Table 2: Growth Performance of *Celosia Argentea* Grown at Different Level of CO_2

Days	Control	0.4mg/g	0.6mg/g	0.8mg/g	1.0mg/g
1	20.35 ± 0.23^{c}	18.45 ± 0.07^{d}	20.35 ± 0.22^{c}	28.25 ± 0.44^{a}	26.35 ± 0.23^{b}
7	34.73 ± 0.04^{c}	29.34 ± 0.13^{d}	28.35 ± 0.22^{e}	42.25 ± 0.43^{a}	39.35 ± 0.22^{b}
14	6.02 ± 0.01^{a}	5.45 ± 0.07^{b}	6.02 ± 0.01^{a}	6.475 ± 0.42^{a}	5.05 ± 0.01^{b}
21	11.51 ± 0.11^{c}	11.35 ± 0.23^{c}	14.95 ± 0.07^{b}	16.03 ± 0.01^{a}	16.05 ± 0.07^{a}

The mean ± standard deviation is represented by the values. Significant ($p<0.05$) values are indicated by a different superscript letter in the same column

Minerals and Nutrients Analysis

Table 3 presents several notable observations regarding the effect of varying carbon dioxide (CO_2) levels regarding the concentrations of different elements in *Celosia argentea* plants. As CO_2 levels increase, Sodium (Na) concentration decreases, while Potassium (K) concentration shows variability, peaking at 1.0 mg/g CO_2. There is a decline in Iron (Fe) concentration, Zinc (Zn) concentration remains consistent across CO_2 levels, and Magnesium (Mg) concentrations are notably lower at higher CO_2 levels. Calcium (Ca) concentration remains relatively stable, with Phosphorus (P) showing a slight decrease, particularly evident at 1.0 mg/g CO_2.

Table 3: Nutrient and Mineral Content of *Celosia Argentea* Leave Grown at Different Levels of CO_2

Parameters	Control	0.4 mg/g	0.6 mg/g	0.8 mg/g	1.0 mg/g
Sodium (ppm)	20.17 ± 0.32^{a}	13.80± 0.01^{c}	17.62±0.01^{b}	10.42±0.13^{d}	15.8 ±0.01^{c}
Potassium (ppm)	86.50 ± 0.02^{b}	57.20 ± 0.01^{d}	85.40±0.43^{c}	76.51±0.01^{d}	113.7±0.01^{a}
Iron (ppm)	1.64 ± 0.03^{a}	0.77 ± 0.01^{b}	1.30 ± 0.01^{a}	0.61 ±0.01^{b}	0.92±0.01^{b}
Calcium (ppm)	20.16 ± 0.01^{a}	20.13±0.02^{a}	19.82±0.01^{b}	19.63±0.02^{b}	20.16±0.01^{a}
Magnesium (ppm)	7.98 ± 0.01^{a}	7.75±0.01^{a}	7.66 ±0.01^{a}	6.82 ±0.06^{b}	7.82 ±0.02^{a}
Phosphorus (ppm)	53.76 ± 0.01^{a}	53.65±0.01^{a}	53.30±0.02^{a}	52.21±0.03^{b}	53.72±0.01^{a}
Zinc (ppm)	3.21 ± 0.02^{a}	3.22 ± 0.01^{a}	3.14 ± 0.01^{a}	3.08±0.01^{a}	3.22 ±0.01^{a}

The mean ± standard deviation is represented by the values. Significant ($p<0.05$) values are indicated by a different superscript letter in the same column

Proximate Analysis

Table 4 illustrates findings from the proximate analysis, revealing distinct responses to varying carbon dioxide (CO_2) levels. Moisture content decreases marginally with increasing CO_2 levels, while ash content shows significant variation, being lowest at 1.0 mg/g CO_2. Carbohydrate content demonstrates a significant rise with increasing CO_2 levels, reaching its peak at 1.0 mg/g CO_2, whereas protein content fluctuates without a discernible trend. Fat content peaks at 1.0 mg/g CO_2, exhibiting notable variability. Fiber content increases notably at 0.8 mg/g and 1.0mg/g CO_2 levels. Pectin content remains relatively stable, while cellulose content shows a slight increase, particularly noticeable at 1.0mg/g CO_2. Finally, Lignin content experiences a slight increase with CO_2 concentration, particularly evident at 1.0 mg/g CO_2.

Table 4: Proximate Analysis of *Celosia Argentea* Leaves Grown at Different Level of CO_2

	Control	0.4 mg/g	0.6 mg/g	0.8 mg/g	1.0 mg/g
Parameters (%)					
MOISTURE	88.07±0.50^{a}	88.25±0.45^{a}	88.67±0.02^{a}	88.36±0.03^{a}	86.46±0.01^{b}
Ash	3.67± 0.01^{a}	2.40±0.04^{b}	2.65± 0.02^{b}	3.05±0.01^{a}	1.92±0.01^{c}
Fat	1.86±0.01^{b}	1.85±0.01^{b}	1.944±0.01^{b}	1.92±0.02^{b}	2.55±0.01^{a}
Fibre	2.13± 0.01^{b}	2.14±0.01^{b}	3.03±0.03^{a}	3.14±0.01^{a}	2.26±0.03^{b}
Protein	2.75± 0.05^{a}	2.53±0.01^{a}	2.45±0.01^{a}	2.34±0.03^{a}	2.75±0.22^{a}
Carbohydrate	1.55± 0.05^{c}	2.77±0.08^{b}	1.28±0.02^{c}	1.189±0.07^{c}	4.115±0.21^{a}
Lignin	12.64±0.01^{b}	12.65±0.01^{b}	12.81±0.01^{b}	13.12±0.01^{a}	12.45±0.01^{b}
Pectin	2.45±0.01^{a}	2.52±0.01^{a}	2.56±0.01^{a}	2.77±0.01^{a}	2.23±0.01^{a}
Cellulose	18.33±0.01^{b}	18.36±0.01^{b}	18.95±0.01^{b}	19.12±0.01^{a}	18.27±0.01^{b}

The mean ± standard deviation is represented by the values. Significant ($p<0.05$) values are indicated by a different superscript letter in the same column

Total Phenol and Total Flavonoid Determination

Table 5 depicts the total amount of phenol and flavonoids in *Celosia argentea* plants across varying carbon dioxide (CO_2) concentrations. Total Phenol content peaks at 0.8 mg/g CO_2, while Total Flavonoid content exhibits a similar trend, indicating a potential influence of CO_2 concentration on secondary metabolite synthesis.

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Table 5: Total Phenol Content, Total Flavonoid Content, 2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Scavenging Ability and 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Scavenging Determination of *Celosia Argentea* Grown at Different Level of CO_2

	Control	0.4mg/g	0.6mg/g	0.8mg/g	1.0mg/g
Total phenol	6.87±0.22^{b}	3.54±0.15^{c}	8.08 ±0.54^{a}	7.99±0.21^{a}	7.77±0.87^{a}
Total flavonoid	1.52±0.12^{c}	0.91±0.11^{d}	2.04±0.07^{b}	2.02±0.02^{b}	2.48±0.04^{a}
ABTS	94.62±1.87^{a}	58.08±3.12^{d}	57.28±0.00^{e}	61.78±0.54^{b}	58.34±4.74^{c}
DPPH	64.58±3.85^{a}	54.44±0.00^{b}	43.49±3.16^{c}	33.46±0.39^{e}	41.63±1.62^{d}

The mean ± standard deviation is represented by the values. Significant ($p<0.05$) values are indicated by a different superscript letter in the same column

Antioxidant Analysis

Table 6 illustrates from the table above (table 5), the varying antioxidant activity levels assessed by ABTS and DPPH then on the figures 1 to 5 are FRAP, ABTS, DPPH, NO, OH, and lipid peroxidation (LPO) illustration levels across different carbon dioxide (CO_2) concentrations. FRAP (Figure 1) and DPPH (Table 5) assays exhibit peak activity at the control, declining with increasing CO_2 levels, whereas the ABTS assay peaks at 0.4 mg/g CO_2 (Table 5). LPO levels fluctuate, initially decreasing at 0.4 mg/g and 0.6 mg/g CO_2, then increasing at 0.8 mg/g, followed by a subsequent decrease at 1.0 mg/g CO_2 (Figure 2), Reduced Glutathione (GSH) levels generally rise with increasing CO_2 concentration (Figure 3). For OH, there's a drastic change from 24.62±4.80e at the control to 80.62±0.01d at 0.4 mg/g CO_2, then a gradual decline as CO_2 levels increases, with the lowest value observed at 1.0 mg/g CO_2 (61.56±0.01a) (Figure 4). On the other hand, for NO, there's a decrease from 63.38±0.11b at the control to 43.00±3.14a at 1.0 mg/g CO_2 (Figure 5).

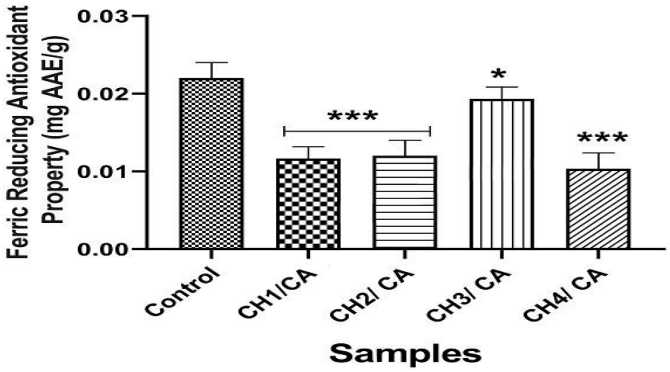

Figure 1: Ferric Reducing Antioxidant Property of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

* ≈ Values are significantly (P<0.05) different from the control

*** ≈ Values are significantly (P<0.001) different from the control

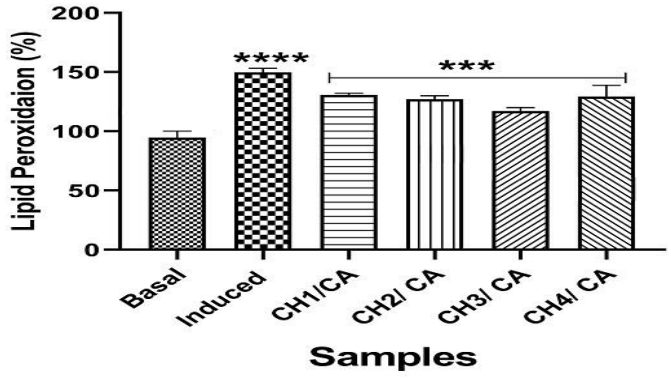

Figure 2: Lipid peroxidation percentage of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

*** ≈ Values are significantly (P<0.001) different from the control

*** ≈ Values are significantly (P<0.0001) different from the control

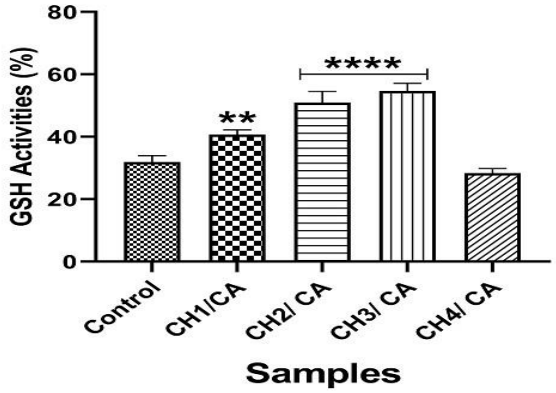

Figure 3: Reduced Glutathione activities percentage of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

** ≈ Values are significantly (P<0.01) different from the control

*** ≈ Values are significantly (P<0.001) different from the control

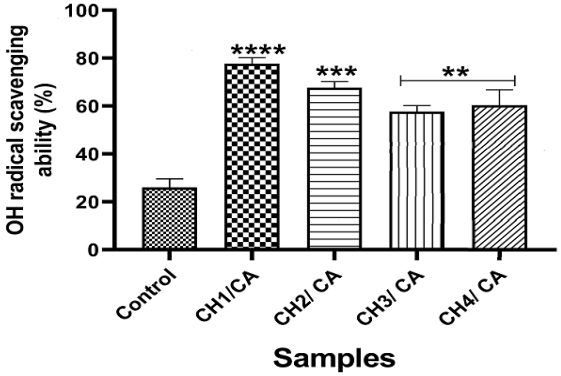

Figure 4: Hydroxyl ion radical scavenging ability of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

** ≈ Values are significantly (P<0.01) different from the control

*** ≈ Values are significantly (P<0.001) different from the control

**** ≈ Values are significantly (P<0.0001) different from the control

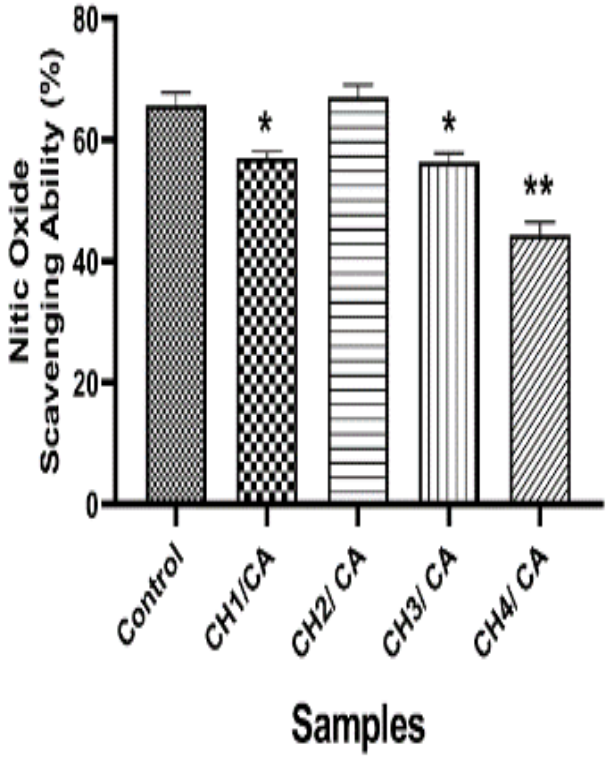

Figure 5: Nitric oxide scavenging ability of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

* ≈ Values are significantly (P<0.05) different from the control

** ≈ Values are significantly (P<0.01) different from the control

Enzyme Analysis

Figures 6, 7, and 8 illustrates the relationship between carbon dioxide (CO_2) levels and the inhibition of alpha-amylase, inhibition of alpha-glucosidase, and inhibition of Angiotensin 1 Converting Enzyme (ACE). As CO_2 levels increase, the percentage inhibition of alpha-amylase and alpha-glucosidase also rises, peaking at 1.0 mg/g CO_2 (Figures 6 & 7). ACE inhibition exhibits fluctuations across CO_2 concentrations, with the highest inhibition noted at 0.8 mg/g CO_2 (Figure 8).

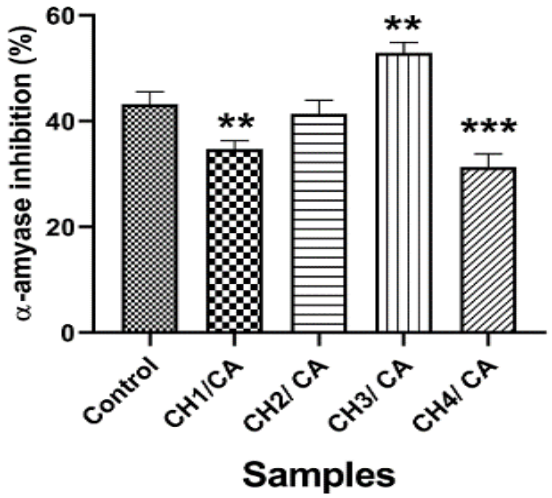

Figure 6: α-amylase inhibition percentage of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

** ≈ Values are significantly (P<0.01) different from the control

*** ≈ Values are significantly (P<0.001) different from the control

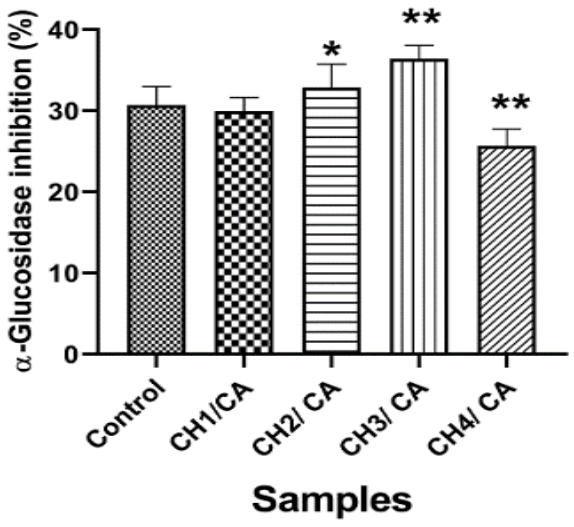

Figure 7: α-glucosidase inhibition percentage of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

* ≈ Values are significantly (P<0.05) different from the control

** ≈ Values are significantly (P<0.01) different from the control

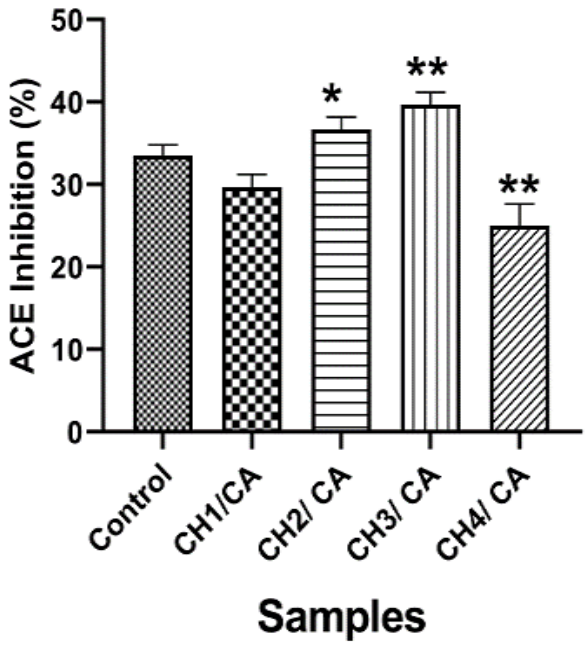

Figure 8: Angiotensin Converting Enzyme inhibition percentage of *Celosia argentea* at various levels of CO_2.

Bars represent mean ± SEM

* ≈ Values are significantly (P<0.05) different from the control

** ≈ Values are significantly (P<0.01) different from the control

DISCUSSION

The result of the research shows that increasing the carbon dioxide exposure to plant can contribute positively to the growth of plant. As observed in Table 1 showing the number of leaves grown at different levels of carbon dioxide (CO_2), initially on Day 1, significant variability in leaf count is evident across different carbon dioxide (CO_2) levels, with the highest leaf counts observed at 0.8mg/g and 1.0mg/g CO_2, suggesting a potential stimulatory effect of elevated carbon dioxide (CO_2) on leaf growth. This trend persists through Day 7, where the highest leaf count consistently appears at 1.0mg/g carbon dioxide (CO_2), followed by 0.8mg/g carbon dioxide (CO_2), indicating a sustained stimulatory impact. Subsequent days demonstrate

intensified differences in leaf count, with consistent superiority of 1.0mg/g carbon dioxide (CO_2), suggesting a sustained benefit of higher carbon dioxide (CO_2) levels on leaf production. This is in correlation with an assessment of tomato plant under raised carbon dioxide and temperature, whereby there was increase in all the growth parameters which later reduced due to the elevated temperature (Rangaswamy *et al.,* 2021).This also corroborates with what was reported about *Pinus radiata* plants to have higher number of leaves under higher carbon dioxide (CO_2) concentration (Conroy *et al.,* 1990). However, it is contrast with what was reported in the studies carried out on *E.americanum* whereby no improvement was observed even when the carbon dioxide (CO_2) level was increased (Gutjahr & Lapointe, 2008). This suggests that the way each plant react to atmospheric conditions may differs (Martinez *et al.,* 2015). Considering Table 2 showing the plant growth, higher carbon dioxide (CO_2) levels, particularly at 0.8mg/g and 1.0mg/g, was observed which demonstrate a consistent stimulatory effect on initial plant growth at Day 1, with subsequent days further reinforcing this trend. By Day 7, the growth enhancement persists, with the highest performance consistently observed at 1.0mg/g carbon dioxide (CO_2). This trend intensifies as the plants mature, with Days 14 and 21 showcasing pronounced differences in growth performance, consistently favoring higher carbon dioxide (CO_2) concentrations. This could be due to enhanced Rubisco carboxylation activity which increases photosynthesis, reduces conductance of the stomata, photorespiration and transpiration (Barnaby *et al.,* 2012; Xu *et al.,* 2015) which can be emphasized by the suggestion that elevated carbon dioxide (CO_2) levels commonly decrease stomata conductance, thereby mitigating plant water stress and potentially resulting in increased leaf size due to enhanced photosynthesis and improved water status (Bunce, 2022; Eichelmann *et al.,* 2004; Riikonen *et al.,* 2005).

Gojon *et al.,* 2023 stated that the adverse effects of elevated carbon dioxide (CO_2) levels (eCO2) on critical physiological processes related to nutrient acquisition and assimilation in C3 plants remain largely unclear (Gojon *et al.,* 2023), this proposes to be true as most the nutrient and mineral content of *Celosia argentea* leaves cultivated under varying carbon dioxide (CO_2) levels decreases. Notable observations include a decrease in Sodium (Na) concentration with increasing carbon dioxide (CO_2) levels, variability in Potassium (K) concentration peaking at 1.0 mg/g carbon dioxide (CO_2), and a decline in Iron (Fe)which correlates with the result of the effect of elevated carbon dioxide (CO_2) on a soybean cultivar whereby reduction in Iron (Fe) was observed. The result of this research shows that Zinc (Zn) concentration remains consistent across carbon dioxide (CO_2) levels which however contradict the soybean cultivar research as Zinc (Zn) was reported to decrease (Köhler *et al.,* 2019) and Magnesium (Mg) concentrations particularly notable at higher carbon dioxide (CO_2) levels. Calcium (Ca) concentration remains relatively stable, while Phosphorus (P) concentration exhibits a slight decrease, notably at 1.0 mg/g carbon dioxide (CO_2) as shown in Table 3.

Furthermore, the proximate analysis (shown in Table 4), observations reveal a slight decrease in moisture content with increasing carbon dioxide (CO_2) levels which is in contrast with the report that atmospheric carbon dioxide (CO_2) does not have any effect on the relative water content of *A. buniifolius* leaf as it was observed that the water content reduced due to drought stress and not increased carbon dioxide (CO_2) (Heck *et al.,* 2022). The ash content varies significantly, being lowest at 1.0 mg/g carbon dioxide (CO_2). Fat content exhibits variability, peaking at 1.0 mg/g carbon dioxide (CO_2). Fiber content increases with higher carbon dioxide (CO_2) levels, notably at 0.8 mg/g and 1.0 mg/g carbon dioxide (CO_2). A report that increased carbon dioxide (CO_2) can reduce the nutritional quality of major crops like rice and wheat by affecting their carbohydrate and protein content due to diminish in the uptake of vital minerals like nitrogen, phosphorus, and iron crucial for plant growth (ScienceDaily, 2022) contradicts as the result of this research shows increase in carbohydrate content significantly raises with increasing carbon dioxide (CO_2) levels, with the highest at 1.0 mg/g carbon dioxide (CO_2) and protein content showing minor fluctuation with no clear trend.

Lignin content slightly increases with carbon dioxide (CO_2) concentration, particularly at 1.0 mg/g carbon dioxide (CO_2). Pectin content remains relatively stable, while cellulose content increases slightly, especially at 1.0 mg/g carbon dioxide (CO_2).

Table 5 show that total phenol content peaks at 0.8 mg/g carbon dioxide (CO_2), while total flavonoid content follows a similar trend, suggesting carbon dioxide (CO_2) concentration influences secondary metabolite synthesis. These findings underscore the sensitivity of phenolic compound production to carbon dioxide (CO_2) levels, implying implications for the medicinal and nutritional value of *Celosia argentea* leaves. Antioxidant activity, assessed by Ferric Reducing Antioxidant Power (FRAP), 2,2'-Azino-bis (3-ethylbenzothiazoline-6 sulfonic acid) (ABTS),2,2-Diphenyl-1-picrylhydrazyl (DPPH), Nitric Oxide (NO) (Figure 4), and Hydroxyl Radical (OH) (Figure 5) assays, varies across carbon dioxide (CO_2) levels, with FRAP (shown in Figure 1) and DPPH assays showing highest activity at the control and declining with increasing carbon dioxide (CO_2), contrasting with ABTS assay, which peaks at 0.4 mg/g carbon dioxide (CO_2).

Lipid Peroxidation (LPO) levels fluctuate across carbon dioxide (CO_2) levels, with a decrease at 0.4 mg/g and 0.6mg/g, followed by an increase at 0.8 mg/g, and a subsequent decrease at 1.0 mg/g (as shown in Figure 2), suggesting a complex relationship between carbon dioxide (CO_2) concentration and lipid peroxidation. Conversely, Reduced Glutathione (GSH) levels as described in Figure 3 generally rise with increasing carbon dioxide (CO_2) concentration, indicating a potential stimulation of antioxidant production under higher carbon dioxide (CO_2) conditions. Results indicate that as carbon dioxide (CO_2) levels increase, the percentage inhibition of alpha-amylase and alpha-glucosidase also increases, peaking at 1.0 mg/g carbon dioxide (CO_2), implying a potential carbon dioxide (CO_2) concentration-dependent enhancement of these inhibitory activities according to Figures 6 and 7. Conversely, Angiotensin 1-Converting Enzyme (ACE) inhibition (shown in Figure 8) displays fluctuation across carbon dioxide (CO_2) concentrations, with the highest inhibition noted at 0.8 mg/g carbon dioxide (CO_2). These findings suggest carbon dioxide (CO_2) concentration's influence on *Celosia argentea* leaves' inhibitory activities, with potential implications for managing conditions like diabetes and hypertension.

CONCLUSION

In summary, this research unveils a nuanced understanding of how elevated carbon dioxide (CO_2) levels intricately shape the growth, nutritional composition, and antioxidant potency of *Celosia argentea* leaves. The observed increase in leaf counts and enhanced growth under higher carbon dioxide (CO_2) concentrations underscores the plant's adaptive response to environmental changes. However, findings reveal a complex interplay between carbon dioxide (CO_2) levels and the nutritional profile of *Celosia argentea*, with notable fluctuations in moisture, ash, fat, fiber, carbohydrate, and protein content across different carbon dioxide (CO_2) concentrations. Additionally, the varied antioxidant activity observed in different assays highlight the multifaceted nature of plant responses to elevated carbon dioxide (CO_2). These results not only provide valuable insights into the physiological mechanisms underlying plant adaptation to changing environmental conditions but also underscore the potential implications for human health and nutrition. Moving forward, subsequent research should focus on in vivo experiments on the effects of leafy tropical vegetation including *Celosia argentea* in order to establish molecular basis of such responses and as a result enhance the utilization of the referred vegetables in sustainable manner to the benefit of environment and mankind.

AUTHORS' CONTRIBUTION

The study was formulated and coordinated by Babatunde S. Ewuloa and Ganiyu Oboh. Olajide R. Ojo and Idowu S. Oyeleye carried out the research, while Stephen A. Adefegha and Olajide R. Ojo processed the experimental data. Patricia

O. Ogundare wrote the first draft of the manuscript, Olajide R. Ojo supervised the manuscript, and Opeyemi B. Ogunsuyi reviewed it.

CONFLICT OF INTEREST

The authors declared that, there is no conflict of interest.

ETHICAL APPROVAL

This study contains ethics of approval and Protocol from by the Federal University of Technology, Akure, School of Agriculture and Agricultural Technology ethical committee (FUTA/SAAT/2020/024).

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