

RESEARCH ARTICLE

# The impact of CO<sub>2</sub> enrichment on fiber dimension and lignocellulose properties of three varieties of kenaf (*Hibiscus cannabinus* L.)

A. Mahdi Khalatbari<sup>1</sup>, H.Z.E. Jaafar<sup>1\*</sup> and A. Ali Khalatbari<sup>1</sup>

<sup>1</sup>Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malasia, 43400

\*Corresponding author: magnificent\_pal@yahoo.com

## Abstract

The effects of two different carbon dioxide levels on fiber yield, fiber dimension and lignocelluloses properties of three varieties of kenaf (*Hibiscus cannabinus* L.) namely Fuhong (FH991), V36 and Kohn-Kaen60 (KK60) were assessed in a growth house experiment at faculty of Agriculture, Universiti Putra Malaysia. Seeds were sown in polyethylene bags containing top (loamy soil). Carbon dioxide enrichment treatment started when the seedlings reached four weeks and plants were exposed to 400 and 800  $\mu\text{mol mol}^{-1}$  of CO<sub>2</sub>. A factorial experiment was arranged in a split plot using a randomized complete block design (The CO<sub>2</sub> chamber is perpendicular to sunrise and sunset) with CO<sub>2</sub> levels as the main plot, and different varieties as sub-plot replicated three times. Different CO<sub>2</sub> levels had significant impact on fiber dimension, fiber yield and lignocellulose properties of bast and core fiber for all three varieties. Result indicated that increasing CO<sub>2</sub> concentration from 400  $\mu\text{mol mol}^{-1}$  to 800  $\mu\text{mol mol}^{-1}$  positively affected fiber of all varieties under study. Increase in fiber length and slight reduction in fiber diameter at 800  $\mu\text{mol mol}^{-1}$  resulted in higher fiber quality for paper production purposes. These results provide significant insights into opportunities for growing of kenaf under enriched CO<sub>2</sub> concentration.

**Keywords:** Carbon dioxide levels, bast fiber, core fiber, fiber dimensions, fiber length, fiber diameter

## 1. Introduction

Carbon dioxide is considered as one of the most prominent and limiting factors in photosynthesis. The CO<sub>2</sub> concentration in atmosphere is currently about 400  $\mu\text{mol}\cdot\text{mol}^{-1}$  and it is expected to increase by 600  $\mu\text{mol}\cdot\text{mol}^{-1}$  CO<sub>2</sub> by the end of 2050 (Taylor and Llyold, 1992). For many years, there have been many interests among scientists about prospect of photosynthesis improvement in crops through CO<sub>2</sub> enrichment (Porter and Grodzinski, 1985). An

increase in plant growth, morphology, development, and yield of many crops has been observed when they were subjected to enriched levels of CO<sub>2</sub>. This type of response depends on CO<sub>2</sub> rate and duration of exposure (Brevoort, 1998). Crops under higher levels of CO<sub>2</sub> acquire positive attributes e.g. an increase in plant height which is important for higher fiber yield and higher photosynthesis rate, with enhanced plant adaptation and growth.

CO<sub>2</sub> is an essential substrate for photosynthesis which links the atmosphere to the biosphere. Enriched CO<sub>2</sub> level would stimulate photosynthesis that will lead to increased carbon (C) uptake and assimilation and eventually results in higher plant growth. Plants with a C3 photosynthetic pathway (like kenaf) often show higher growth rate compared to those with a C4 pathway (Amthor and Loomis, 1996; Bowes, 1993; Poorter *et al.*, 1992; Rogers *et al.*, 1996). Considering C3 plants, positive responses at ambient CO<sub>2</sub> levels are mainly related to competitive inhibition of photorespiration by CO<sub>2</sub> and the internal CO<sub>2</sub> concentrations of C3 leaves due to being less than the Michaelis-Menton constant of ribulose biphosphate carboxylase/oxygenase (Amthor and Loomis, 1996). Many reports have stated increased photosynthesis under elevated CO<sub>2</sub>, this increase varies for plants with a C3 (33% to 40% increase) versus a C4 (10% to 15% increase) (Kimball and Idso, 1983; Prior *et al.*, 2003).

Elevated CO<sub>2</sub> can change C partitioning/allocation beside photosynthesis and aboveground growth stimulation. Increased C supply which is caused by elevated atmospheric CO<sub>2</sub> could profoundly induce the distribution of photosynthate belowground (Ceulemans and Mousseau, 1994; Prior *et al.*, 1997; Rogers *et al.*, 1996). It was observed in many cases that plants tend to allocate photosynthate to tissues which is needed in order to acquire the most limiting resources such as water and nutrients (Chapin *et al.*, 1987).

It has been reported from many studies that there would be lower stomata conductance and lower transpiration rate due to higher CO<sub>2</sub> concentration. (Rogers and Dahlman, 1993). In fact, improved plant water relations were observed by both C3 and C4 species. Elevated CO<sub>2</sub> induces the partial closure of leaf stomatal guard cells that results in slowing transpiration (Jones and Mansfield, 1970). Experiments and studies conducted in growth chambers and glasshouses under controlled condition have indicated that enriched CO<sub>2</sub> declines transpiration for C3 plants (Allen *et al.*, 1994; Jones *et al.*, 1984, 1985). Reduction in transpiration rate and an increase in photosynthesis can contribute to

improve the water use efficiency the ratio of carbon fixed to water transpired, (Baker *et al.*, 1990; Morison, 1985; Sionit *et al.*, 1984) as it was observed in this experiment on young kenaf varieties.

Kenaf plant absorbs CO<sub>2</sub> from the atmosphere more than any other crop, about 1.5 tons of CO<sub>2</sub> seems sufficient in order to produce 1 ton of dry matter of Kenaf. It means that each hectare of Kenaf consumes 30-40 t of CO<sub>2</sub> per growing cycle (Kimball and Idso, 1983). Growth of kenaf is precisely indispensable due to its importance for fiber production and fiber content. The most prominent advantage of CO<sub>2</sub> enrichment is the vegetative growth of young plants (Kimball and Idso, 1983). The greenhouse industry has proven the advantages of manipulating CO<sub>2</sub> and light in order to benefit proper grown crops in controlled conditions for extended periods. When plants are young, they grow swiftly and somehow exponentially while older plants grow more in a linear way (Lindhout and Pet, 1990).

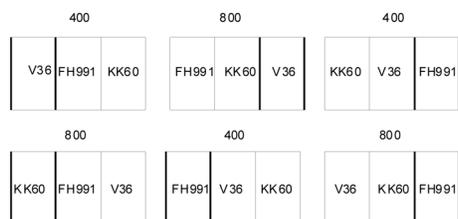
Due to lack of documentation on the impact of CO<sub>2</sub> enrichment on growth and fiber development of kenaf and knowing that CO<sub>2</sub> enrichment studies need optimization of the plant microclimatic conditions such as temperature, relative humidity, irrigation and nutrients, the present study is important in order to gain needed information on fiber development of kenaf under CO<sub>2</sub> enrichment. The objective of this study was i): to evaluate the growth and histochemical responses of selected varieties of kenaf under different CO<sub>2</sub> concentration and ii): to choose the best variety and best CO<sub>2</sub> level to improve the fiber quality.

## 2. Materials and Methods

### 2.1. Experimental location, plant materials and treatments

The experiment was carried out under growth house at Ladang 2, Faculty of Agriculture Glasshouse Complex, University Putra Malaysia (longitude 101°

44° N and latitude 2° 58'S, 68 m above sea level) with a mean atmospheric pressure of 1.013 kPa. One-month old kenaf seedlings of three varieties namely Fuhong (FH991), V36 and Kohn-Kaen60 (KK60) were selected for this experiment. Seeds were obtained from the Institute of Tropical Forestry and Forest products. Seeds were sown in polyethylene bags with 16 cm diameter and 24 cm height containing approximately 12.5 kg of top soil (loamy soil-fine loamy, siliceous, isohyperthermic). Carbon dioxide enrichment treatment started when the seedlings reached four weeks old and the plants were exposed to 400 and 800  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub>. The experiment was arranged in a split plot using a randomized complete block design with CO<sub>2</sub> levels being the main plot, and varieties as sub-plot replicated three times. Each treatment consisted of twelve seedlings (Figure 1).



**Figure 1.** Experimental layout using nine mini structure as the main plot of, with 400 = 400  $\mu\text{mol mol}^{-1}$  and 800 = 800  $\mu\text{mol mol}^{-1}$ . The sub-plot consist of three varieties namely Fuhong (FH991), V36 and Kohn-Kaen60 (KK60) . Each colum with CO<sub>2</sub> level represents a block.

### 2.2. Growth house microclimate and CO<sub>2</sub> enrichment treatment

The seedlings were kept in specially constructed growth houses receiving 12-hour photoperiod and average photosynthetic photon flux density of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Daily and night temperatures were recorded at 30±1.0°C and 20±1.5°C, respectively, and relative humidity at about 70% to 80%. Vapor pressure

deficit ranged from 1.01 to 2.52 kPa. Carbon dioxide at 99.8% purity was supplied from a high-pressure CO<sub>2</sub> cylinder and injected through a pressure regulator into fully sealed 2 m x 3 m growth houses at 2-hour daily and applied continuous from 8 to 10a.m. (Ibrahim et al., 2010). The CO<sub>2</sub> concentration at different treatments was measured using Air Sense™ CO<sub>2</sub> sensors (high-pressure CO<sub>2</sub> cylinder) designated to each chamber during CO<sub>2</sub> exposition period. Plants were irrigated three times a day for 5 min using drip irrigation with emitter capacity of 2 L hr<sup>-1</sup>. The experiment lasted 10 weeks since CO<sub>2</sub> treatment was applied.

### 2.3. Fibre dimensions and values

Stem samples for the fiber studies were obtained from the second internode counting from the base. Small slices or slivers (fiber bundles) were obtained from the bast and from the central wood core were macerated in 67% HNO<sub>3</sub> and then (suspended) boiled in water bath for 5 minutes (the time of boiling has been modified from 10 minutes to 5 minutes in order to prevent any fiber slivers deterioration). Fiber suspension that had been dyed with toluidine was finally placed on microscope slides. Fiber samples were teased on slides and viewed under calibrated microscope (MEIJI® light microscope with Nikon® camera attached) and following fiber dimensions were recorded. A total of eight fibers were measured for each sample and mean values calculated for fiber length (fl), fiber diameter (fd), fiber lumen diameter (fld) and fiber wall thickness. The optimum fiber length for bast is with range of 1.8 to 4.0 mm and this range for core is 0.4 to 1.0 mm. Considering fiber diameter, for bast it is within the range of 14 - 27  $\mu\text{m}$  and for core is 18 - 37  $\mu\text{m}$  (fwt) (Ogbonnya et al., 1997).

From these above measurements, the following values were calculated: coefficient of suppleness (CS) or flexibility coefficient as fld/fd (Petri, 1952), slenderness ratio (SR) as fl/fd (Rydholm, 1967) and runkel ratio (RR) as 2 x fwt/fld, (Okereke, 1962).The runkel ratio <1 indicates high fiber quality. Lower runkel ratio (> 1) indicates that fiber has lower value

of cell wall thickness and fiber lumen diameter which is a positive sign of high quality fiber.

#### 2.4. Chemical analysis

The proportions of chemical properties in these three varieties of kenaf bast and core fibres were determined based on approved standard of Technical Association of Pulp and Paper Industry (TAPPI standard). The percentage of lignin was determined by TAPPI standard T 222 os-74 using 72% sulphuric acid. For holocellulose, it was based on the method of Wise *et al.*, (1946) using sodium chlorite, 10% acetic acid and acetone with a slight modification. The percentage of  $\alpha$ -cellulose was determined by TAPPI standard T 203 os using 8.3% NaOH, 17.5% NaOH and 2N acetic acid.  $\alpha$ -cellulose extractive was derived from holocellulose.

#### 2.5. Experimental design and statistical analysis

The factorial experiment was arranged in a split plot using a randomized complete block design (RCBD) with CO<sub>2</sub> levels being the main plot, and varieties as subplot replicated three times. Each treatment consisted of twelve seedlings. Data were analysed using analysis of variance by Statistical Analysis System computer package (SAS Institute Inc., 2007). Mean separation test between treatments was performed using Duncan New Multiple Range Test (DNMRT) and p-value of  $\leq 0.05$  was regarded as significant.

### 3. Results

#### 3.1. Fiber dimension

The effect of different CO<sub>2</sub> and different varieties levels on the fiber dimensional properties of three kenaf varieties are presented in Table 1. Different levels of CO<sub>2</sub> had significant impact on fiber dimension of bast and core fiber. Different varieties showed significant effect on fiber attributes except for core lumen diameter and core cell wall thickness. The interaction between different varieties and

different CO<sub>2</sub> levels had no significant impact on fiber dimension parameters except for bast lumen diameter and core fiber diameter (Table 1).

The highest bast fiber length was recorded for 800  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub> level with value of 3.10 mm whereas CO<sub>2</sub> level of 400  $\mu\text{mol mol}^{-1}$  recorded bast fiber length of 2.68 mm (Table 2). Higher bast fiber diameter, bast lumen diameter and bast cell wall thickness were recorded at ambient CO<sub>2</sub> level (400  $\mu\text{mol mol}^{-1}$ ) with value of 23  $\mu\text{m}$ , 10.1  $\mu\text{m}$  and 4.1  $\mu\text{m}$  respectively. CO<sub>2</sub> of 800  $\mu\text{mol mol}^{-1}$  had bast fiber diameter of 21.3  $\mu\text{m}$ , bast lumen diameter of 8.9  $\mu\text{m}$  and bast cell wall thickness of 3.5 (Table 2). For core fiber attributes, the highest core fiber length were at elevated CO<sub>2</sub> (800  $\mu\text{mol mol}^{-1}$ ) with value of 0.98 mm whereas at ambient CO<sub>2</sub> the core fiber length was 0.92 mm (Table 2). It was observed that CO<sub>2</sub> level of 400  $\mu\text{mol mol}^{-1}$  attained higher core fiber diameter of 24.3  $\mu\text{m}$ , core lumen diameter of 16.9  $\mu\text{m}$  and core cell wall thickness of 3.7  $\mu\text{m}$  compared to elevated CO<sub>2</sub> (Table 2). These results for bast and core fiber diameter indicated that elevated CO<sub>2</sub> of 800  $\mu\text{mol mol}^{-1}$  would produce higher fiber and this is because of higher fiber length and lower fiber diameter when the plants are subjected to CO<sub>2</sub> enrichment.

Considering the impact of different varieties on fiber dimension attributes, variety FH991 recorded the highest bast fiber length with value of 3.1 mm, followed by varieties V36 and KK60 with bast fiber length of 2.8 mm and 2.7 mm, respectively (Table 3). Variety FH991 also attained the highest bast fiber diameter of 23.0  $\mu\text{m}$ . Variety KK60 presented a bast fiber diameter of 22.0  $\mu\text{m}$ , followed by variety V36 with value of 21.4  $\mu\text{m}$  (Table 3). For core fiber length and diameter, variety FH991 recorded the highest core fiber length of 0.96 mm and core fiber diameter of 23.0  $\mu\text{m}$ . For core fiber length and core fiber diameter, there was no significant difference between varieties V36 and KK60 (Table 3). Variety V36 attained core fiber length of 0.95  $\mu\text{m}$  whereas variety KK60 had value of 0.94  $\mu\text{m}$ . For core fiber diameter variety V36 and KK60 presented similar values of 21.8  $\mu\text{m}$  (Table 3).

**Table 1.** ANOVA split-plot arrangement based on randomized complete block designed for bast and core fiber measurements of three varieties of kenaf namely: FH991, V36 and KK60 under different CO<sub>2</sub> levels (400 and 800 μmol mol<sup>-1</sup>).

SOV	d.f	Bast fibre length	Bast fiber diameter	Bast lumen diameter	Bast cell wall thickness	Core fibre length	Core fiber diameter	Core lumen diameter	Core cell wall thickness
<b>Block</b>	2	0.06 <sup>ns</sup>	0.66 <sup>ns</sup>	0.30 <sup>ns</sup>	0.19 <sup>ns</sup>	0.0001 <sup>ns</sup>	6.68**	0.40 <sup>ns</sup>	0.16*
<b>A(CO<sub>2</sub> levels)</b>	1	0.79**	12.60**	5.72**	1.55**	0.01**	74.33**	18.93**	0.98**
<b>Block*A</b>	2	0.007	0.27	0.17	0.08	0.00001	0.69	0.05	0.07
<b>B(Varieties)</b>	2	0.20**	3.83**	15.47**	2.72**	0.0009**	2.65*	0.09 <sup>ns</sup>	0.04 <sup>ns</sup>
<b>AxB</b>	2	0.06 <sup>ns</sup>	1.75 <sup>ns</sup>	2.34*	0.09 <sup>ns</sup>	0.0001 <sup>ns</sup>	5.03**	0.27 <sup>ns</sup>	0.03 <sup>ns</sup>

\*\* : Significant at ( $p \leq 0.01$ ), \* : Significant at ( $p \leq 0.05$ ), ns: not significant

**Table 2.** Bast and core fiber dimensions of three different kenaf varieties (FH991, V36 and KK60) under different CO<sub>2</sub> levels (400 and 800 μmol mol<sup>-1</sup>).

CO <sub>2</sub> LEVELS	Bast fibre length (mm)	Bast fiber diameter (μm)	Bast lumen diameter (μm)	Bast cell wall thickness (μm)	Core fibre length (mm)	Core fiber diameter (μm)	Core lumen diameter (μm)	Core cell wall thickness (μm)
<b>400 (μmol mol<sup>-1</sup>)</b>	2.68 <sup>b</sup>	22.99 <sup>a</sup>	10.05 <sup>a</sup>	4.09 <sup>a</sup>	0.92 <sup>b</sup>	24.28 <sup>a</sup>	16.87 <sup>a</sup>	3.73 <sup>a</sup>
<b>800 (μmol mol<sup>-1</sup>)</b>	3.10 <sup>a</sup>	21.32 <sup>b</sup>	8.92 <sup>b</sup>	3.51 <sup>b</sup>	0.98 <sup>a</sup>	20.22 <sup>b</sup>	14.82 <sup>b</sup>	3.27 <sup>b</sup>

Means in the same row followed by different letters indicate a significant difference according to DMRT ( $p \leq 0.05$ ).

### 3.2. Fiber values

The effects of different CO<sub>2</sub> levels on the fiber derived values of three kenaf varieties are presented in Table 4. For bast fiber, slender ratio was affected only by CO<sub>2</sub> levels. The runkle ratio and coefficient of suppleness were affected by varieties and the interaction between varieties and CO<sub>2</sub> levels. For core fiber, slender ratio showed significant difference for CO<sub>2</sub> levels and the interaction between varieties and CO<sub>2</sub> levels. Varieties, CO<sub>2</sub> and the interaction between treatments had

significant impact on core coefficient of suppleness. Core runkle ratio showed no significant difference among all treatments (Table 4).

Considering the interaction of varieties with levels of CO<sub>2</sub> in this experiment, FH991 under CO<sub>2</sub> concentration of 400 μmol mol<sup>-1</sup> recorded the highest coefficient of suppleness for bast (52.2) which was followed by interaction of FH991 with CO<sub>2</sub> level of 800 μmol mol<sup>-1</sup> (45.20) and KK60 subjected to 800 μmol mol<sup>-1</sup> (43.9).

**Table 3.** Bast and core fiber length and diameter of three different kenaf varieties (FH991, V36 and KK60) under different CO<sub>2</sub> levels (400 and 800 μmol mol<sup>-1</sup>).

Varieties	Bast fibre length (mm)	Bast fibre diameter (μm)	Core fibre length (mm)	Core fibre diameter (μm)
FH991	3.09 <sup>a</sup>	23.01 <sup>a</sup>	0.96 <sup>a</sup>	23.02 <sup>a</sup>
V36	2.83 <sup>b</sup>	21.43 <sup>b</sup>	0.95 <sup>b</sup>	21.87 <sup>b</sup>
KK60	2.74 <sup>b</sup>	22.02 <sup>b</sup>	0.94 <sup>b</sup>	21.85 <sup>b</sup>

Means in the same row followed by different letters indicate a significant difference according to DMRT ( $p \leq 0.05$ ).

**Table 4.** ANOVA split-plot arrangement based on randomized complete block designed (RCBD) for bast and core fiber ratios measurement of three varieties of kenaf namely: FH991, V36 and KK60 under different CO<sub>2</sub> levels (400 and 800 μmol mol<sup>-1</sup>).

SOV	d.f	Bast slender ratio	Bast coefficient of suppleness	Bastrunkle ratio	Core slender ratio	Core coefficient of suppleness	Core runkle ratio
Block	2	210.73 <sup>ns</sup>	1.15 <sup>ns</sup>	0.002 <sup>ns</sup>	20.65*	41.35**	0.004 <sup>ns</sup>
A(CO <sub>2</sub> levels)	1	3668.07**	13.35 <sup>ns</sup>	0.01 <sup>ns</sup>	474.35**	60.28**	0.00001 <sup>ns</sup>
Block*A	2	23.98	1.34	0.004	0.96	8.19	0.001
B(Varieties)	2	154.85 <sup>ns</sup>	186.14**	0.35**	0.78 <sup>ns</sup>	11.15*	0.0007 <sup>ns</sup>
AxB	2	151.91 <sup>ns</sup>	50.85*	0.02*	19.91*	68.17**	0.0003 <sup>ns</sup>

\*\* : Significant at ( $p \leq 0.01$ ), \* : Significant at ( $p \leq 0.05$ ), ns: not significant

The lowest bast coefficient of suppleness was recorded for V36 exposed to CO<sub>2</sub> level of 800 μmol mol<sup>-1</sup> that was 36.3. This is due to lower lumen diameter compared to fiber diameter (Table 5). Variety FH991 under ambient CO<sub>2</sub> level (400 μmol mol<sup>-1</sup>) and elevated CO<sub>2</sub> level (800 μmol mol<sup>-1</sup>) presented the lowest runkle ratio of 0.56 and 0.52. The highest runkle ratio belonged to variety KK60 subjected to CO<sub>2</sub> level of 400 μmol mol<sup>-1</sup> (Table 5). The highest core slender ratio was recorded by varieties FH991, KK60 and V36 subjected to elevated

CO<sub>2</sub> with ratio of 49.9, 49.2 and 46.9 respectively whereas variety FH991 under ambient CO<sub>2</sub> level of 400 μmol mol<sup>-1</sup> had the lowest core slender ratio of 36.3 (Table 5). Variety KK60 under 800 μmol mol<sup>-1</sup> attained the highest core coefficient of suppleness of 76.0 followed by variety FH991 under CO<sub>2</sub> concentration of 800 μmol mol<sup>-1</sup> with coefficient of suppleness of 74.4. The lowest suppleness ratio belonged to varieties FH991 (65.8) and KK60 (69.6) when these varieties were subjected to 400 μmol mol<sup>-1</sup> (Table 5).

**Table 5.** Bast and core fiber dimensions of three different kenaf varieties (FH991, V36 and KK60) under different CO<sub>2</sub> levels (400 and 800  $\mu\text{mol mol}^{-1}$ ).

Treatments	Bast coefficient of suppleness	Bastrunkle ratio	Core slender ratio	Core coefficient of suppleness
Fh991(400 $\mu\text{mol mol}^{-1}$ )	52.18 <sup>a</sup>	0.52 <sup>a</sup>	36.31 <sup>c</sup>	65.83 <sup>c</sup>
Fh991(800 $\mu\text{mol mol}^{-1}$ )	45.20 <sup>b</sup>	0.56 <sup>a</sup>	49.85 <sup>a</sup>	74.41 <sup>a</sup>
V36 (400 $\mu\text{mol mol}^{-1}$ )	39.05 <sup>cd</sup>	0.98 <sup>c</sup>	40.53 <sup>b</sup>	73.93 <sup>a</sup>
V36 (800 $\mu\text{mol mol}^{-1}$ )	36.33 <sup>d</sup>	0.97 <sup>c</sup>	46.87 <sup>a</sup>	69.91 <sup>b</sup>
KK60 (400 $\mu\text{mol mol}^{-1}$ )	39.38 <sup>cd</sup>	1.05 <sup>c</sup>	38.25 <sup>bc</sup>	69.58 <sup>c</sup>
KK60 (800 $\mu\text{mol mol}^{-1}$ )	43.91 <sup>bc</sup>	0.86 <sup>b</sup>	49.17 <sup>a</sup>	76.01 <sup>a</sup>

Means in the same row followed by different letters indicate a significant difference according to DMRT ( $p \leq 0.05$ ).

**Table 6.** ANOVA split-plot arrangement based on randomized complete block designed (RCBD) for bast and core fiber lignocelluloses attributes of three varieties of kenaf namely: FH991, V36 and KK60 under different CO<sub>2</sub> levels (400 and 800  $\mu\text{mol mol}^{-1}$ ).

SOV	d.f	Bast holocellulose	Bast $\alpha$ -cellulose	Bast lignin	Core holocellulose	Core $\alpha$ -cellulose	Core lignin
Block	2	0.22 <sup>ns</sup>	0.81 <sup>ns</sup>	0.007 <sup>ns</sup>	0.51*	0.22 <sup>ns</sup>	0.02 <sup>ns</sup>
A(CO <sub>2</sub> levels)	1	11.36**	7.73**	3.38**	12.83**	9.97**	12.16**
Block*A	2	0.18	0.35	0.04	0.17	0.10	0.31
B(Varieties)	2	0.06 <sup>ns</sup>	0.12 <sup>ns</sup>	0.10 <sup>ns</sup>	0.54*	0.04 <sup>ns</sup>	0.11 <sup>ns</sup>
AxB	2	0.32 <sup>ns</sup>	0.05 <sup>ns</sup>	0.13 <sup>ns</sup>	0.23 <sup>ns</sup>	0.04 <sup>ns</sup>	0.01 <sup>ns</sup>

\*\* : Significant at ( $p \leq 0.01$ ), \* : Significant at ( $p \leq 0.05$ ), ns: not significant

### 3.3. Chemical properties

The effects of different CO<sub>2</sub> levels on the fiber chemical properties of three kenaf varieties are presented in Table 6. Bast and core lignocellulose properties of three kenaf varieties were affected by different CO<sub>2</sub> concentrations. Varieties had no significant impact on these properties except for core holocellulose. There was no significant interaction observed for lignocellulose properties (Table 6).

Chemical composition of kenaf lignocellulose would give great deal of information on feasibility of plant material for paper making purposes. The highest bast and core lignocelluloses were observed for plants under elevated CO<sub>2</sub> (Table 7). The highest bast holocellulose,  $\alpha$ -cellulose and lignin were observed at enriched CO<sub>2</sub> of 800  $\mu\text{mol mol}^{-1}$  (87.3%, 57.9% and 14.3% respectively), whereas the lowest holocellulose of 85.75,  $\alpha$ -cellulose of 56.54 and lignin of 13.45 were recorded for plants exposed to 400  $\mu\text{mol mol}^{-1}$  (Table 7).

**Table 7.** Bast and core lignocellulose of three different kenaf varieties (FH991, V36 and KK60) under different CO<sub>2</sub> levels (400 and 800 μmol mol<sup>-1</sup>).

CO <sub>2</sub> LEVELS	Bast holocellulose	Bast α-cellulose	Bast lignin	Core holocellulose	Core α-cellulose	Core lignin
400 μmol mol <sup>-1</sup>	85.75 <sup>b</sup>	56.54 <sup>b</sup>	13.45 <sup>b</sup>	82.55 <sup>b</sup>	46.03 <sup>b</sup>	19.95 <sup>b</sup>
800 μmol mol <sup>-1</sup>	87.34 <sup>a</sup>	57.85 <sup>a</sup>	14.32 <sup>a</sup>	84.24 <sup>a</sup>	47.52 <sup>a</sup>	21.60 <sup>a</sup>

Means in the same row followed by different letters indicate a significant difference according to DMRT ( $p \leq 0.05$ ).

**Table 8.** ANOVA split-plot arrangement based on randomized complete block designed for bast, core and total fiber yield of three varieties of Kenaf namely: FH991, V36 and KK60 under different CO<sub>2</sub> levels of 400 and 800 (μmol mol<sup>-1</sup>) at the end of experiment period.

SOV	d.f	Bast fiber yield	Core fiber yield	Total fiber yield
<b>Block</b>	2	0.003n.s	0.01**	0.04*
<b>A(CO<sub>2</sub> levels)</b>	1	4.15**	13.78**	33.05**
<b>Block*A</b>	2	0.001	0.00001	0.002
<b>B(Varieties)</b>	2	0.84**	4.06**	8.56**
<b>AxB</b>	2	0.03**	0.21**	0.35**

\*\* : Significant at ( $p \leq 0.01$ ), \* : Significant at ( $p \leq 0.05$ ), ns: not significant.

### 3.4. Fiber yield

The bast, core and total fiber yield of the three kenaf varieties were affected by CO<sub>2</sub> concentrations and varieties. The interaction between varieties and CO<sub>2</sub> level had significant impact on bast, core and total fiber yield (Table 8).

The optimum value of bast fiber yield was obtained by FH991 when it was subjected to enriched CO<sub>2</sub> level of 800 μmol mol<sup>-1</sup> (14.3 g plant<sup>-1</sup>) followed by variety V36 (14.0 g plant<sup>-1</sup>) and KK60 (13.5 g plant<sup>-1</sup>) at the end

of experimental period while the lowest value for bast fiber yield was recorded by variety KK60 under ambient CO<sub>2</sub> level of 800 μmol mol<sup>-1</sup> with value of 12.58 g plant<sup>-1</sup> (Table 9).

The highest core fiber yield of 24.2 g plant<sup>-1</sup> was recorded by FH991 under elevated CO<sub>2</sub>, followed by variety V36 with value of 23.2 g plant<sup>-1</sup> and variety KK60 with value of 22.5 g plant<sup>-1</sup>. KK60 under ambient CO<sub>2</sub> level attained the lowest value 20.6 g plant<sup>-1</sup>. Varieties V36 and FH991 under ambient CO<sub>2</sub> level showed a core fiber yield of 21.9 g plant<sup>-1</sup> and 22.2 g plant<sup>-1</sup> respectively (Table 9).

**Table 9.** Bast, core and total fiber yield of three different kenaf varieties (FH991, V36 and KK60) under different CO<sub>2</sub> levels (400 and 800  $\mu\text{mol mol}^{-1}$ ).

Treatments	Bast fibre yield (g plant <sup>-1</sup> )	Core fibre yield (g plant <sup>-1</sup> )	Total fibre yield (g plant <sup>-1</sup> )
FH991(400 $\mu\text{mol mol}^{-1}$ )	13.18 <sup>d</sup>	22.21 <sup>d</sup>	35.39 <sup>d</sup>
FH991(800 $\mu\text{mol mol}^{-1}$ )	14.33 <sup>a</sup>	24.17 <sup>a</sup>	38.49 <sup>a</sup>
V36 (400 $\mu\text{mol mol}^{-1}$ )	13.12 <sup>d</sup>	21.89 <sup>e</sup>	35.01 <sup>e</sup>
V36 (800 $\mu\text{mol mol}^{-1}$ )	13.97 <sup>b</sup>	23.21 <sup>b</sup>	37.18 <sup>b</sup>
KK60 (400 $\mu\text{mol mol}^{-1}$ )	12.58 <sup>e</sup>	20.57 <sup>f</sup>	33.15 <sup>f</sup>
KK60 (800 $\mu\text{mol mol}^{-1}$ )	13.48 <sup>c</sup>	22.54 <sup>c</sup>	36.02 <sup>c</sup>

Means in the same row followed by different letters indicate a significant difference according to DMRT ( $p \leq 0.05$ ).

Enriched CO<sub>2</sub> level of 800  $\mu\text{mol mol}^{-1}$  increased total fiber yield measured for all varieties. Variety FH991 subjected to elevated CO<sub>2</sub> level showed the highest total fiber yield of 38.5 g plant<sup>-1</sup>, followed by varieties V36 with total fiber yield of 37.2 g plant<sup>-1</sup> and KK60 with value of 36.0 g plant<sup>-1</sup>. All varieties under ambient CO<sub>2</sub> attained lower total fiber yield. Variety KK60 presented a total fiber yield of 33.2 g plant<sup>-1</sup> which was the lowest value followed by varieties V36 (35.0 g plant<sup>-1</sup>) and variety FH991 (35.4 g plant<sup>-1</sup>) (Table 9).

#### 4. Discussion

One factor to increase fiber production and quality is to speed up the growth process (Ogbonnya *et al.*, 1997). There is no documentation on the impact of enriched CO<sub>2</sub> level on physiological aspects and lignocelluloses attributes of kenaf as a fibrous plant. This could be plausible by using carbon dioxide at different concentrations (400 and 800  $\mu\text{mol mol}^{-1}$ ) at seedling growing stage.

It was observed that most of fiber dimension and lignocellulose attributes were influenced by the CO<sub>2</sub> concentrations of different varieties. These parameters showed higher value when plants were subjected to elevated CO<sub>2</sub> concentration of 800  $\mu\text{mol mol}^{-1}$ . Fiber dimension parameters showed higher quality for paper production purposes when all three varieties were under enriched CO<sub>2</sub> level. The first quantitative review of crop yield based on responses to elevated CO<sub>2</sub> was conducted by Kimball and Idso, (1983). He reported an average increase of 33% among 37 agricultural species, which included fiber, root/tuber, and leaf crops. Cure and Acock, (1986) also collected data on total yield of 10 major crops (grain, leaf, tuber and fiber) and found an average increase of 41% when CO<sub>2</sub> concentration was doubled.

Plants show many differences, namely morphology, variation in photosynthesis, respiration, biochemical composition and even at reproductive phase when are subjected to different concentrations of CO<sub>2</sub>. Plants with C3 pathway like kenaf have two different physiological processes which are directly affected:

photosynthesis and transpiration. Net photosynthesis rate increases as CO<sub>2</sub> concentration increase, probably due to a decrease in photorespiration and partly due to an increase in substrate supply. A lower stomatal conductance will reduce transpiration rate as well (Cure and Acock, 1986; Amthor and Loomis, 1996). One of possible reasons of increase in photosynthesis rate is the lower of water plant losses that leads to higher growth rate. Most of plants exposed to higher CO<sub>2</sub> concentrations have plant growth stimulation (Kimball and Idso, 1983). There have been reports on cotton (*Gossypium hirsutum* L.) as a good source of fiber production in which elevated CO<sub>2</sub> level (almost twice of ambient) enhanced total biomass and harvestable yield (Reddy *et al.*, 1999; Tischler *et al.*, 2000). One of the most important benefits of elevated CO<sub>2</sub> is in increment of leaf gas exchange capacity, especially when plants are under undesirable climatic conditions (Mark and Jackson, 2000).

In present experiment kenaf varieties under enriched CO<sub>2</sub> level had higher fiber yield and support the result of Mark and Jackson (2000). Enhancement of plant growth and fiber yield subjected to enriched CO<sub>2</sub> has been reported before (Norby *et al.*, 1996; Ceulemans and Mousseau, 1994; Idso and Idso, 1994; Saxe *et al.*, 1998). This is due to an increase in total leaf area and leaf photosynthetic rate coupled with a decline in shoot respiration rate (Ceulemans and Mousseau, 1994). It has been stated that the relative area of mesophyll cells is more closely related to photosynthesis rate than are the epidermis and the vascular bundles (Parkhurst, 1986; Evans, 1999; Roderick *et al.*, 1999).

It has been reported by Ceulemans and Mousseau, (1994) and Norby *et al.*, (1999) that enriched CO<sub>2</sub> level often results in increasing in the total leaf area, leaf weight and leaf weight compared to shoot weight. Leaf area development is a key factor for determination of total plant productivity and could be different in a range of environmental conditions (Taylor and Llyold, 1992). Total leaf area of kenaf varieties especially FH991 under elevated CO<sub>2</sub> of 800  $\mu\text{mol mol}^{-1}$  had higher value compared to the plants subjected to ambient CO<sub>2</sub>

(400  $\mu\text{mol mol}^{-1}$ ) (data not shown). This higher total leaf area probably led to a higher total biomass and total fiber yield. Many studies have reported that crop growth and yield are favored by elevated CO<sub>2</sub> (Kimball and Idso, 1983; Amthor and Loomis, 1996). Elevated atmospheric CO<sub>2</sub> would increase leaf and canopy photosynthesis, especially in C3 crops like kenaf and this is the reason why the presence of CO<sub>2</sub> is not enough to saturate Rubisco. Higher CO<sub>2</sub> concentration can inhibit the competing process of photorespiration by suppressing RuBP oxygenase activity increasing carbon assimilates for plant growth and development (Lawlor and Mitchell, 2000).

There has been a debate that CO<sub>2</sub> may also have impact on cell soluble/fiber composition of plant tissue. Plant with higher growth rate would have higher fiber fractions, whereas increasing in carbon assimilation may result in higher cell soluble content due to storage of excess carbohydrates. (Akin *et al.*, 1994, 1995; Owensby *et al.*, 1996; Frehner *et al.*, 1997; Carter *et al.*, 1999; Fritschi *et al.*, 1999). Morgan *et al.*, 2004 stated that plant subjected to elevated CO<sub>2</sub> concentration would obtain larger yield which is an indication of greater plant growth. Higher plant growth can result in a higher lignin component. This statement is in accordance with our findings in which kenaf varieties under enriched CO<sub>2</sub> level recorded higher total biomass and therefore higher fiber yield and greater lignocelluloses properties compared to plants under ambient CO<sub>2</sub> of 400  $\mu\text{mol mol}^{-1}$ . But in other studies conducted in other microclimate, small impact of CO<sub>2</sub> on cell solubles, hemicellulose–cellulose, lignin and fiber fractions was observed (Akin *et al.*, 1994, 1995; Soussana and Loiseau, 1997; Booker, 2000; Fritschi *et al.*, 1999).

## 5. Conclusion

A doubling ambient CO<sub>2</sub> concentration leads to higher fiber content for kenaf (as a C3 plant and fibrous plant, responds strongly to elevated CO<sub>2</sub> level) that could be highly considered for paper production.

Varieties subjected to elevated CO<sub>2</sub> level attained higher value for fiber dimension (especially fiber length) and lignocelluloses properties that are prominent attributes related to paper producing quality. Among varieties, it was variety FH991 that attained the highest values for most attributes. Based on this study result, increment in fiber length and slight reduction in fiber diameter caused by enriched CO<sub>2</sub> level of 800 μmol mol<sup>-1</sup> will result in higher fiber quality for paper production purposes.

### Acknowledgment

The authors gratefully acknowledge Universiti Putra Malaysia (UPM ) their contribution for this project number of 5523867 FRGS (Fundamental Research Grant Scheme).

### References

- Allen, L.H., Valle, R.R. Jr., Mishoe, J.W., Jones, J.W. 1994. Soybean leaf gas-exchange responses to carbon dioxide and water stress. *Agron. J.* 86, 625–636.
- Amthor, J.S., Loomis, R.S. 1996. Integrating knowledge of crop responses to elevated CO<sub>2</sub> and temperature with mechanistic simulation models: Model components and research needs. In: Koch, G.W., Mooney, H.A (eds). *Carbon dioxide and terrestrial ecosystems*. Academic Press., San Diego, CA, pp: 317–346
- Baker, J.T., Allen, L.H., Boote, K.J. Jr., Jones, P., Jones, J.W. 1990. Rice photosynthesis and evapotranspiration in subambient, ambient, and superambient carbon dioxide concentrations. *Agron. J.* 82, 834–840.
- Bowes, G. 1993. Facing the inevitable: Plants and increasing atmospheric CO<sub>2</sub>. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44, 309–332.
- Brevoort, P. 1998. The blooming United State botanical market: A new overview. *Herbalgram.* 44, 33–46.
- Ceulemans, R., Mousseau, M. 1994. Effects of elevated atmospheric CO<sub>2</sub> on woody plants. *New Phytol.* 127, 425–446.
- Chapin, F.S., Bloom, III, A.J. Field, C.B., Waring, R.H. 1987. Plant responses to multiple environmental factors. *Bioscience.* 37, 49–55.
- Ibrahim, M.H., Jaafar, H.Z.E., Haniff, M.H., Raffi, M.Y. 2010. Changes in growth and photosynthetic patterns of oil palm seedling exposed to short term CO<sub>2</sub> enrichment in a closed top chamber. *Acta Physiol. Plant.* 32, 305–313.
- Jaafar, H.Z.E. 1995. Impact of Environmental Stress on Reproductive Development in Sweet pepper (*Capsicum annuum*). Ph.D. Thesis, University of Nottingham, Nottingham, UK.
- Jones, P., Allen, L.H., Jones, J.W. Jr., Boote, K.J., Campbell, W.J. 1984. Soybean canopy growth, photosynthesis, and transpiration responses to whole-season carbon dioxide enrichment. *Agron. J.* 76, 633–637.
- Jones, P., Allen, L.H., Jones, J.W. Jr., Valle, R. 1985. Photosynthesis and transpiration responses of soybean canopies to short- and long-term CO<sub>2</sub> treatments. *Agron. J.* 77, 119–126.
- Jones, R.J., Mansfield, T.A. 1970. Increases in the diffusion resistances of leaves in a carbon dioxide-enriched atmosphere. *J. Expt. Bot.* 21, 951–958.
- Kimball, B.A., Idso, S.B. 1983. Increasing atmospheric CO<sub>2</sub>: Effects on crop yield, water use, and climate. *Agr. Water Manage.* 7, 55–72.
- Lindhout, P., G. Pet. 1990. Effects of CO<sub>2</sub> enrichment on young Plant growth of 96 genotypes of tomato (*Lycopersicon esculentum*). *Euphytica.* 51, 191–196
- Morison, J.I.L. 1985. Sensitivity of stomata and water use efficiency to high CO<sub>2</sub>. *Plant Cell environment.* 14, 467 – 474.

- Ogbonnaya, C.I., Nwalozie, M.C., Roy-Macauley, H., Annerose, D.J.M. 1997. Growth and water relations of Kenaf (*Hibiscus cannabinus* L.) under water deficit on a sandy soil. *Ind. Crops Prod.* 8, 65–76.
- Okereke, O.O. 1962. Studies on the fiber dimensions of some Nigerian timbers and raw materials. Part 1: Research Report No. 16: Fed. Ministry of Commerce and Industry Lagos Nigeria.
- Petri, R. 1952. Pulping studies with African tropical woods. *TAPPI* 35, 157-160.
- Poorter, H. Gifford, R.G., Kridelman, P.E., Wong, S.E. 1992. A quantitative analysis of dark respiration and carbon content as factors in the growth response of plants to elevated CO<sub>2</sub>. *Australian Journal Botany.* 40, 501 – 513.
- Porter, M.A., Grodzinski, B. 1985. CO<sub>2</sub> enrichment of protected crops. *Hort. Res.* 7, 345–398.
- Prior, S.A., Runion, G.B., Mitchell, R.J., Rogers, H.H., Amthor, J.S. 1997. Effects of atmospheric CO<sub>2</sub> on longleaf pine: Productivity and allocation as influenced by nitrogen and water. *Tree Physiol.* 17, 397–405.
- Prior, S.A., Torbert, H.A. Runion, G.B., Rogers. H.H. 2003. Implications of elevated CO<sub>2</sub> induced changes in agroecosystem productivity. *J. Crop Prod.* 8, 217–244.
- Rogers, H.H., Dahlman, R.C. 1993. Crop responses to CO<sub>2</sub> enrichment. *Vegetatio.* 105, 117–131.
- Rogers, H.H., Prior, S.A. Runion, G.B., Mitchell, R.J. 1996. Root to shoot ratio of crops as influenced by CO<sub>2</sub>. *Plant Soil.* 187, 229–248.
- Rydholm, S.A. 1967. *Pulping process.* New York, Wiley and Sons, 1167p.
- Sellers, T., Miller, G.D., Fuller, M.J., 1993. Kenaf core as a board raw material. *Forage Prod. J.* 43, 69-71.
- SAS. 2007. *SAS/STAT User’s Guide, Version 9.2.* SAS Institute Inc., Cary, NC, USA.
- Sionit, N., Rogers, H.H., Bingham, G.E., Strain, B.R. 1984. Photosynthesis and stomatal conductance with CO<sub>2</sub>-enrichment of container- and field-grown soybeans. *Agron. J.* 76, 447–451.
- Taylor, J.A., Llyold, J. 1992. Sources and sinks of atmospheric CO<sub>2</sub>. *Aust. J. Bot.* 40, 401–418.
- Wise, L.E., Murphy, M., D’Addieco, A.A. 1946. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and studies on the hemicelluloses. *Pap. Trade J.* 122(2), 35-43.