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RESEARCH PAPER

Management Factors Affecting Nitrogen Content, Cell Wall Thickness and the Fiber Diameter of the Kenaf Fibers (*Hibiscus cannabinus* L.)

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A B S T R A C T:

Two field tests have done in 2013 and 2014 on two kenaf varieties: Fuhong-952 and 4383 at University Putra Malaysia which was to investigate the effect of preharvest factors of nitrogen content in the cell wall of the fibers. Different types and doses of fertilizers were used as preharvest factors, which was potassium as muriate of potash at the rate of (0.0, 100 and 150 kg/ha), was added to the plants in two different growth stages, halve dose at planting day and others' after planting by three weeks. At the second time of application boron and zinc added at (0.0, 1.0, 1.5 and 0.0, 5.0 kg/ha, of borax and zinc chloride), respectively. Besides that, during the second field test just FH-952 as plant material was selected, other levels of zinc were added which was to display its impact of quality characteristics. The best result of nitrogen content (4.82%) was achieved for FH-952 when potassium was added at the high level, nevertheless 4383 was when potassium, boron and zinc were applied at the rate of (150, 1.0 and 5.0) kg/ha, respectively by (3.33%). Changing of nitrogen content caused to increase cell wall thickness, so the biggest result of the cell wall thickness was recorded again when potassium was added alone at a high level (150) kg/ha, which was by (7.73 µm) of FH-952 variety. K, Z and combined treatments; K×Z, K×B, and K×B×Z were significantly affected fiber diameter. During the second experiment zinc performances caused to achieve tough fibers which was possible for using as biocomposite materials.

KEY WORDS: *Hibiscus cannabinus* L., Fertilizer, Fiber Morphology, Fiber Improving.
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1. INTRODUCTION:

Natural plant fibers occupy an important position as material for the textile and engineering applications. Kenaf (*Hibiscus cannabinus* L.) is one of the natural fibers belongs to Malvaceae family. Quality characteristics were focused to improve on kenaf fibers during current project. The stem of kenaf is divided into two different fibers such as: the bast fiber and inner woody core fiber. Chemical and morphological properties of these fibers are entirely different. The average of 35 % bast in the stem, and the woody part is the remaining one. The bast fiber of the kenaf is longer and slender with higher cellulose content than the core fiber, whereas the core fiber of the stem of the kenaf plant is shorter and wider with higher lignin content (Khalil *et al.,* 2010; Mossello *et al.,* 2010).

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Scientists are trying to find out the best ways to improve the quality and quantity of this crop, since kenaf fibers can be used as alternative material in biocomposite and textile applications, it also environmental friendly. Alexopoulou *et al.* (2013) stated that the kenaf stems are used in biocomposites and insulation mat, and in textile applications. Thereby, importance of this crop is increased over the world. Through this current study spent a lot of effort to find the best way to progress not just quantity but also quality of the fibers. For this purpose, chemical contents in the cell wall of the fibers were cared, and also thought about how to change the amount of the chemicals. Nitrogen or protein content was one of these chemicals existing in the cell wall of the kenaf fiber. Even so, the average of protein around the cell walls of fiber crops is low compare to other chemicals particularly cellulose, but might be small changing of nitrogen or protein average cause to improve quality of the fiber.

The main product of kenaf bast fibers is paper, while the whole plant has high protein and good digestibility and may be palletized (Webber III and Bledsoe, 1993). Chemical contents such as: hemicellulose, cellulose, lignin, protein, and pectin can be caused to improve mechanical and morphological characteristics of the plant fibers. Decided to use different amount of fertilizers to change the average of protein content of the plant cell walls which was due to the fiber quality can be enhanced by using fertilizers. Liu *et al.* (2013) stated that fertilizers intensively affect the yield and quality of bast fiber crops. Increasing the production is not enough but rather, the quality should be improved. Previous studies have mentioned many factors which can affect the production and quality of kenaf fiber such as: cultivars, plant breeding, cultivation practices, fertilization, plant population, climate, and others. Therefore, it is necessary to choose the most economically best way. For any crop, potassium is one of the essential elements for various growth stages.

Van Brunt and Sultenfuss (1998) stated that potassium fertilizer improves physical quality, resists illness, and increases crop yield and quality. The nitrogen and potassium application have increased the ramie yield (*Boehmeria nivea*). While, nitrogen, phosphorus, and potassium nutrients were not enough for the flax, but also the micronutrients are necessary. The fiber content in flax stem was increased, and the absorption of other nutrients was also improved when boron was applied (Schumann and Sumner, 2004). Panhwar *et al.* (2011) showed a positive effect of boron and zinc application along with other macronutrients for maize plants.

As known, fertilizers can be added to the plants in wide range without having strong details about its effect on end uses of plant production which was especially in developing countries. This current study will try to display the effect of potassium, boron and zinc on improving quality of fiber crops as a guide for the above purpose.

2. MATERIALS AND METHODS

2.1. Materials

The Institute of Tropical Forestry and Forest Production (INTROP) at the University Putra Malaysia provided seeds of two new kenaf varieties, Fuhong-952 (FH-952) and 4383. The FH-952 variety originated in Fujian Fuzhou, China, and the 4383 variety was first introduced in Bangladesh. Selecting varieties is the initial and most important step in cultivation. The FH-952 and 4383 varieties were selected based on a previous success studies by INTROP and were found suitable for tropical climates.

2.2. Location

Both experiments were conducted at Taman Pertanian University, Seksyen Tanaman Ladang Kongsi Petak C, University Putra Malaysia, Serdang, Selangor, Malaysia (2^{o}59’11.5”N, 101^{o}42’ 29.9”E, 50 m a.s.l.).

2.3. Soil Sampling

Random soil samples were taken at depths of 0 to 15 cm from several places on the land before it was divided into plots. The samples were then transported to the laboratory. Next, the soil was air-dried and sieved through a 2 mm pore size sieve. Table 1 presents the chemical and physical properties of the soil.

Table 1. The initial physical and chemical properties of the soil used in the experiment

Soil properties											
Chemical properties											
C	N	pH	EC	P	K	Ca	Mg	Mn	Cu	Zn	Fe
%			µS/ cm	µg/g							
1.45	0.10	6.00	108.50	1.56	80.17	451.10	95.76	7.26	ND	0.93	73.30
Physical properties											
Sand	Silt	Clay									
%											
46.07	9.92	43.92									

ND= not detected

experiment also the land divided to three blocks each block content 5 plots (treatments), so the total number in this experiment were 15 plots (treatments). Following treatments were used in the first experiment V1 and V2 represent FH-952 and 4383 varieties, respectively, while K1, K2, and K3 represent potassium levels (0.0, 100, and 150) kg/ha, respectively. B1, B2, and B3 represent boron levels (0.0, 1.0, and 1.5) kg/ha, respectively, while Z1 and Z2 represent zinc levels (0.0 and 5) kg/ha, respectively. While in the second experiment the plots were treated with different levels of zinc (Z1 to Z5) which were (0.0, 1.5, 3.0, 4.5, and 6.0) kg/ha, respectively. The plot size was 1m^2, each plot content of 66 plants. The distance between plants was 5cm while between row to row just 30cm.

Nitrogen content as chemical compound, cell wall thickness and fiber diameter as morphological parameters were taken in the current study. Morphological properties of the bast kenaf fiber were determined after preparing the fiber through maceration process. The samples of the bast fibers were taken from the middle of the stem. The process of maceration was carried out based on the practical association of pulp and paper industry (TAPPI) standard T233-Su-64 (Smook, 2002). After preparation the slides, the measurement of bast fibers for fiber morphology was carried out by Quantimeter Image Analyzer Microscope (Nikon DS-Ri1/ECLIPSE E200).

2.5. Statistical Analysis

Data on nitrogen content and morphological properties were subjected to Analysis of Variance (ANOVA) by using SAS Statistics 9.3 (2002-2010). Student-Newman Keuls (SNK) at $P \leq 0.05$ was used to perform the mean comparison.

3. RESULTS AND DISCUSSION

3.1. Nitrogen Content

Fig. 1 shows the results of nitrogen content (%) were achieved in this study. Results indicate that these treatments seem to be significantly different from others; T13 K3B1Z1, T14 K3B3Z2 and T16 K3B2Z2 on nitrogen content of kenaf fibers. Potassium has contributed a valuable improvement for both varieties. However, potassium performance on FH-952 variety was better than Bangladesh variety 4383.

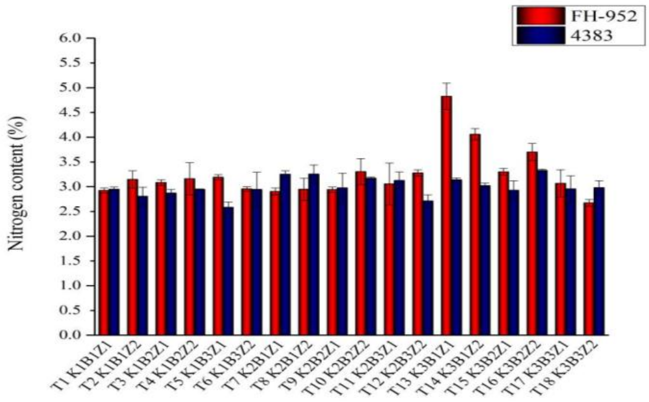

Figure 1: Combined effect of potassium, boron, and zinc on the nitrogen content (%) in FH-952 and 4383 varieties.

By increasing the rate of potassium, the nitrogen content was increased. For instance, when potassium was added at the rate of 150 kg/ha (T13 K3B1Z1), the nitrogen content increased to 4.82% for FH-952 variety as compared to control treatment (T1 K1B1Z1), the amount of nitrogen content was only 2.92% (Fig. 1). Thus, these findings is in accordance with Hossain *et al.* (2011) and Igras and Danyte (2007) who reported that potassium fertilizer significantly affects many physiological and biochemical processes, and it eventually plays important role in these processes in plants. Alexopoulou *et al.* (2007) also reported that high level of nitrogen fertilizer increased the amount of nitrogen content (%) in both stem parts (bast and core) fibers of kenaf plant.

On the other hand, potassium with boron, boron with zinc, and potassium, boron and zinc had also positively affected the nitrogen content in both fractions of kenaf fibers (bast and core), (Fig. 1). Normally, boron has two important roles. First, it assists to uptake nutrients from soil to plants and second, it recovers the growth of crops. Consequently, it is considered as important nutrient for plants (Salih *et al.*, 2014). Initially, for FH-952 variety, when boron was added at the rate of 1 kg/ha, the value of nitrogen content was 3.08% and after increased to 1.5 kg/ha, the value of nitrogen content was also increased to 3.19%. Additionally, this result is in agreement with (Kipriotis *et al.*, 2007). They found that nitrogen content of leaves and stems was affected by kenaf varieties and fertilizers.

In contrast, the value of nitrogen content in 4383 variety dropped when treated with boron as compared to the control treatment. Probably, these rates of boron are toxic to this variety. The fact can be seen in treatments 1, 3, and 5 (Fig. 1). Previous researcher, Hardy *et al.* (2003) revealed that the excessive rate of boron can be toxic to plants. Fundamentally, the main interaction between fertilizers potassium, boron and zinc with varieties was significant. Conversely, the interaction between varieties with zinc, and varieties with boron and zinc were not significant.

Zinc has not significantly affected the nitrogen content of kenaf fibers. (Chaney, 1993) found similar results and stated that when heavy metals exceeded in plants, it strongly affects the photosynthesis process. Subsequently, if the level of zinc increases in plants, phytotoxicity can occur. Zinc has no significant effect on increasing nitrogen content as well. Therefore, some fluctuations between both level of 0 and 5 kg/ha realized a big gap to be explored. These facts show that may zinc plays a role in developing nitrogen content. Nevertheless, different levels are required for different plants particularly when it is used along with potassium and boron. Significant effect was observed when zinc was interacted at the rate of 5 kg/ha with potassium at the rate of 150 kg/ha during treatment 14 as shown in (Fig. 1). The value of nitrogen content in this condition was 4.06 %. The finding is supported by Bell and Dell (2008) who stated that the average of protein changed due to zinc fertilizer because it plays an imperative role for this purpose.

These results are also in agreement with Hossain *et al.* (2011) who reported that the rate of major and minor minerals changed evidently with kenaf plant components and five kenaf varieties; V36, G4, KK60, HC2, and HC95. Furthermore, they stated that G4 and HC2 varieties have the highest nitrogen content in the stem respectively.

Despite that, related literature emphasized on zinc application as essential for crops. Shivay *et al.* (2015) and Prasad (2003) reported that zinc application is important since it not only increases the zinc concentration and which is not only needed by rice, but it also increases the protein content of rice kernels. Some other researchers also found that zinc has antioxidative capacity which reduces cadium toxicity and protects macromolecules like proteins and enzymes from cadmium toxicity (Aravind and Prasad, 2004; Aravind and Prasad, 2003; Köleli *et al.*, 2004; Prasad, 2003).

While, zinc levels significantly affected fiber diameter and cell wall thickness during second experiment (Table 3 and Fig. 5).

3.2. Improve Quality Properties

On the other hand, some scientists claimed that zinc has physiological functions such as production of energy and protein; eventually, it is a vital nutrient (Andreini *et al.*, 2006; Gupta *et al.*, 2008; Hänsch and Mendel, 2009; Mousavi *et al.*, 2013; Qiao *et al.*, 2014). However, Marschner (1997) reported that zinc is a crucial inorganic nutrient and a cofactor of over 300 enzymes and proteins involved in cell division, nucleic acid metabolism, and protein synthesis. Hence, this study and previous research supports the claim that zinc is beneficial for plant growth and quality improvement.

Proteins in this case, are polymers of amino acids which can improve fiber quality. On the other hand, it is considered as one of the important chemicals in the structure of the fiber cell walls. Almost all biochemical activities, including growth and development are controlled by proteins.

As it is shown from fig. 2 improve quality properties of kenaf fiber due to effect of potassium, and also in what way potassium supports of photosynthesis process, and how its cause to change nitrogen content (protein) which improves quality of fiber production.

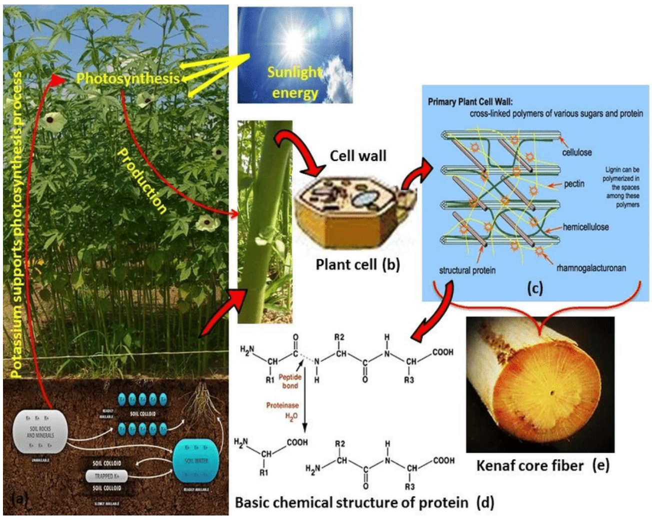

Figure 2: Effect of potassium in supporting photosynthesis process on changing the nitrogen content (protein) which improves cell walls and then quality of fiber production (Salih, 2015).

Image (a) from fig. 2 shows the available forms of potassium for plants in the soil (www.cropnutrition.com). As stated earlier, potassium is taken up by plants in large quantities and it is necessary to many plant functions, including: carbohydrates metabolism, enzyme activation, osmotic regulation, and protein synthesis. Potassium is also essential for photosynthesis, for nitrogen fixation, starch formation, and translocation of sugars. Besides that, image (b) shows the cell wall of the plant, and then cross-linked polymers of various sugars and protein of the primary plant cell wall is shown in Image (c), (Koning, 1994). On the other hand, image (d) shows basic chemical structure of protein (Donohue and Osna, 2003). In the image (c) can see structural of protein existed between cellulose of the plant cell wall. The change of protein average around the cell wall might be caused to cell division, and then it causes to improve quality production as can see in (Image e).

Through looking of image (c) also can get to other important fact about the structure of plant cell walls which believed that, it helps scientists to more understand about plant cell walls. Many people know that the cell wall is made of layers of variously arranged/aligned cellulose microfibrils, but this polymer of glucose is not the only wall element by any means. In addition to cellulose, walls have a range of various polymers of sugars and sugar-derivatives including: hemicellulose, rhamnogalacturonan, pectin and structural protein (Koning, 1994).

Adair and Snell (1988) reported that cell walls in plants carry small amount of protein whereby, primary walls contain more protein than secondary walls. This present study revealed that the amounts of protein in the cell walls of the kenaf fibers can be improved, and eventually, the quality and quantity of the fiber can be affected based on amount of nitrogen available in the cell wall systems. Many researchers stated that the potassium fertilization is a main essential nutrient for protein synthesis and supports photosynthesis process. Lubin and Ennis (1964) stated that the RNA synthesis occurs due to protein synthesis, and that potassium performs important function in protein synthesis. Thus, the importance of potassium cannot be overemphasized.

Hence, it can also be anticipated if some plants have high concentration of potassium during their life cycle it might have affected their nitrogen content. It was discovered that in kenaf FH-952 variety, the stem parts (bast and core fibers) carry more nitrogen content compared to the 4383 variety. It was recorded the amount as 4.82% and 3.33%, equal to 30.15% and 20.79% protein, respectively (Fig. 1). Results are supported by Berardo (1992) stated that both of protein and fiber content were significant differences according of cultivars.

Findings revealed that different response of plant nutrients uptake might cause to differentiate nitrogen content. In control treatments, both FH-952 and 4383 have lower nitrogen content value at 2.92% and 2.95% only (Fig. 1). This was incorporated with Webber (1993) who found that crude protein is different among the six kenaf varieties and plant parts when they were planted between 1989 to 1990. Moreover, Hossain *et al.* (2011) also reported that the nitrogen content varies between plant fractions for five different varieties of kenaf.

3.3. Cell Wall Thickness

Chemicals in the cell wall of the fibers have effects on the quality properties. A cellulose average of kenaf fibers is about 60-80%. In addition, lignin and moisture are around 5-20% and 20%, respectively (Coates, 1996; Mohamed *et al.*, 1995; Nishimura *et al.*, 2002). Fig. 3 shows the schematic representation of the plant cell wall, where it explains the layers of the cell wall with contents.

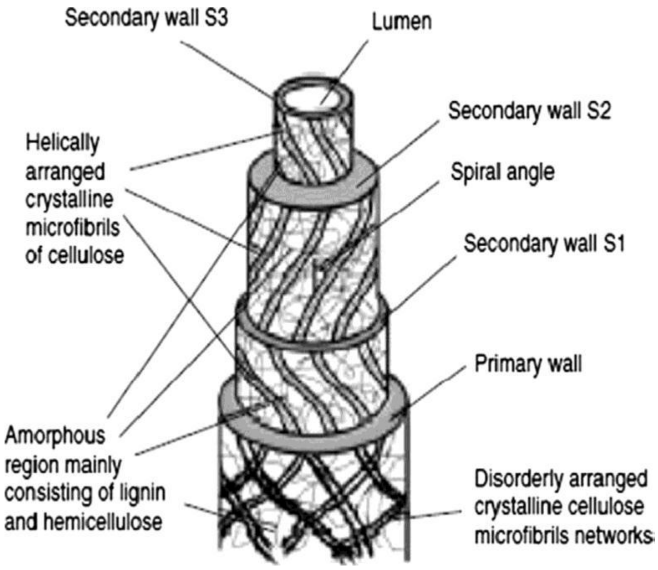

Figure 3: Schematic image of cell wall of the natural plans (Rouison *et al.*, 2004).

Generally, boron alone, variety with boron have not affected the fiber diameter and cell wall thickness. Despite that, the biggest result of the cell wall thickness was recorded when potassium was added alone at a high level (T13 K3B1Z1), which was for FH-952 variety (Table 2 and Fig. 4).

Table 2. Analysis of variance (ANOVA) for the effect of different treatments and their interactions on the fiber diameter and cell wall thickness of bast fiber during the first experiment

Source	DF	Fiber Diameter (μm)		Cell wall thickness (μm)	
		Mean S	F.Value	Mean S	F.Value
V**	1	0.0003	0.00^{ns}	0.00467	0.03^{ns}
K	2	73.230	78.60^{*}	11.1068	65.63*
B	2	2.2077	2.37^{ns}	0.22561	1.33^{ns}
Z	1	6.4094	6.88^{*}	1.45371	8.59^{*}
V×K	2	24.891	26.71^{*}	3.23206	19.10^{*}
V×B	2	0.4168	0.45^{ns}	0.41435	2.45^{ns}
V×Z	1	10.163	10.91^{*}	1.84868	10.92^{*}
K×B	4	9.1702	9.84^{*}	1.42814	8.44^{*}
K×Z	2	1.5194	1.63^{ns}	0.93634	5.53^{*}
B×Z	2	7.6707	8.23^{*}	1.33862	7.91^{*}
K×B×Z	4	7.8599	8.44^{*}	0.96564	5.71^{*}
V×K×B	4	9.3924	10.08^{*}	1.38581	8.19^{*}
V×K×Z	2	3.4760	3.73^{*}	1.68653	9.97^{*}
V×B×Z	2	6.4698	6.94^{*}	0.65091	3.85^{*}
V×K×B×Z	4	13.902	14.92^{*}	1.75423	10.37^{*}

* significant at 5% level, ns = not significant
** V= Variety, K= Potassium, B= Boron and Z= Zinc

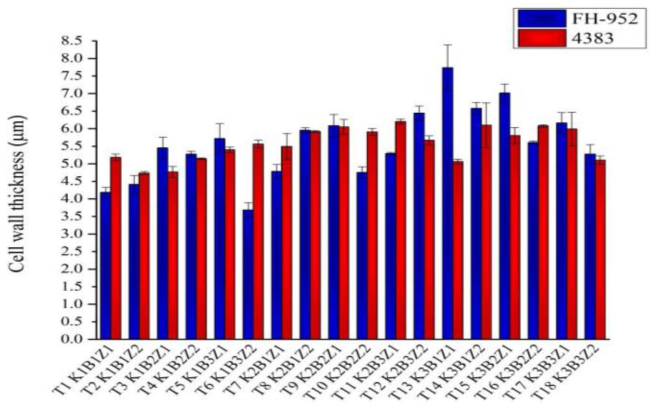

Figure 4: Combined effect of potassium, boron and zinc on cell wall thickness (μm) in FH-952 and 4383 varieties.

During the second experiment, Zn levels affected negatively on the cell wall thickness, since with increasing the levels of Zn the cell wall thickness was reduced and being thinners. Table 3 and Fig. 5 show the effect of zinc levels on cell wall thickness of the bast kenaf fiber. The biggest cell wall was recorded as 7μm in control treatment when Zn was not added to the cultivated plants in the soil. When fiber has the big cell wall thickness, it leads to a reduction of the lumen width.

Table 3. Analysis of variance (ANOVA) for the effect of zinc on the fiber diameter and cell wall thickness of bast fiber during the second experiment

Source	DF	Fiber Diameter (μm)		Cell wall thickness (μm)	
		Mean S	F. Value	Mean S	F Value
R**	2	0.52	2.85^{ns}	0.74	4.01^{ns}
Z	4	35.42	191.86^{*}	1.17	6.33*

* Significant at 5% level, ns = not significant
** R = Replication, Z = Zinc

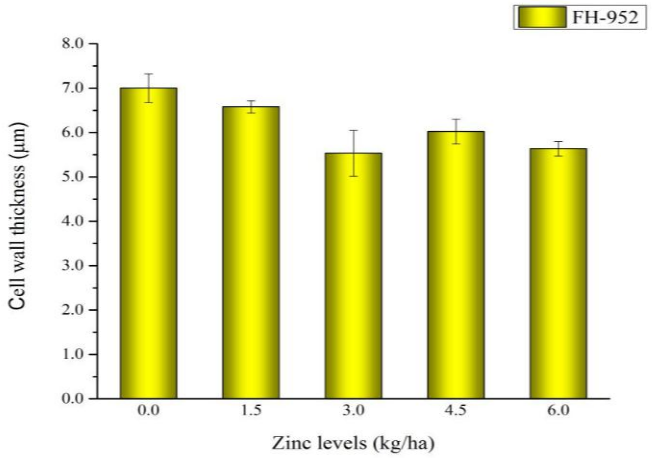

Figure 5: Effect of zinc on cell wall thickness (μm) for kenaf FH-952 variety.

Generally, results from this section of the study are supported by Norton *et al.* (2006). They stated that the chemical composition and structural organization of the cell wall polymers are affected by many factors which further affected the properties of the plant fibers.

Besides of cell wall thickness the fiber dimeter was affected by fertilizers as can be seen in some images microscopy of the bast fiber of both kenaf varieties; FH-952 and 4383 during both experimental trials (Fig. 6). The images explicate the differences between fibers affected by fertilizers and varieties. And also, more details about this morphological property can be found in (Table 2 and 3).

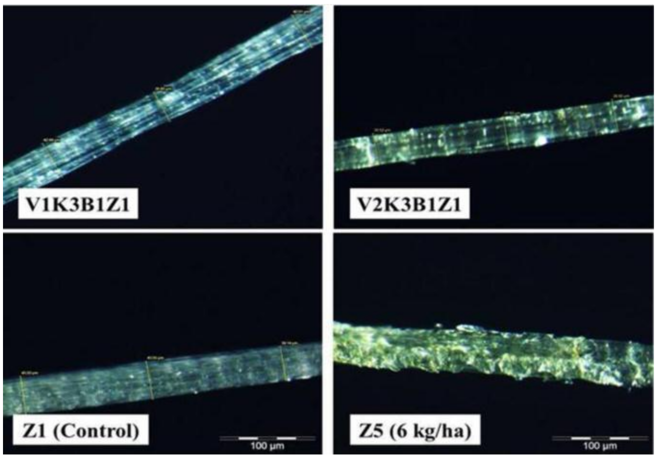

Figure 6: Image microscopy of fiber diameter of kenaf bast fiber. V1 = FH- 952 variety, V2 = 4383 variety, B1 and Z1 = Boron and Zinc were controlled, K3 and Z5 = 150 and 6.0 kg/ha, respectively.

4. CONCLUSIONS

From the study, it was found that quality of production has a good relationship with the amount of chemical compounds in the cell wall structures of the fiber. The chemicals which mainly extracted from fertilizers would cause tremendous changes in the amount of the chemicals in the cell wall of the fiber.

This study has shown that plant requests to add potassium and micronutrients (boron and zinc) at different amounts to improve kenaf fiber quality. Also, results have shown that the potassium at the rate of 150 kg/ha significantly affects the plants. It improves important chemical composition (protein content) in the cell walls of the kenaf fibers bast and core together. Then, the importance of boron and zinc were followed to potassium also to improve fiber production by increasing nitrogen content (protein). On the other hand, the performance of FH-952 variety to improve fiber production through increasing protein content was clearer than the 4383 variety.

Finally, the purpose to obtain the best value of nitrogen content was showed that the fertilizer applications combined or separated were more important to decide which fibers suited to use as biocomposite and which one for textile and other industrial applications. Additionally, in both experiments the fiber diameter was also significantly changed by adding single and combined fertilizers especially that affected refers to potassium and zinc. This present study found that 4383 variety more possible to use as biocomposite purposes than FH-952 due to its fiber too hardness while flexibility of FH-952 causes to use in the textile section.

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Conflict of Interest

The authors declare no conflict of interest.

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