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RESEARCH PAPER

Nutritional Value of Different Kenaf Leaves (*Hibiscus cannabinus* L.) Varieties Enhanced by Using Different Concentrations of Humic Acid

Deedar Maghded Sultan[1,2*] ,Rabar Fatah Salih[1]

1 Department of Filed Crops, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, Kurdistan Region, Iraq
2 Department of Engineering, West Erbil Emergency Hospital, General Directorate of Health-Erbil, Ministry of Health, Kurdistan Region Government-Iraq

A B S T R A C T:

Nutritive value of kenaf leaves encourages researchers to do researching, due to its distinction of some areas like; medicinal plant, source of food for human and as feedstock for animals. The objective of the study is to improve nutritive components of kenaf leaves from different varieties by adding some concentrations of humic acid. The kenaf varieties were FH952, HC95 and HC2 as (V1, V2 and V3), respectively, and humic acid concentrations were 0.0, 0.5, 1.0, and 1.5 g/4L water m^{-2} as (H0, H1, H2 and H3), respectively. The experiment was conducted at two locations; Grdarasha Field and Ainkawa Research Station in summer season 2021. The results indicated that the FH952 variety recorded highest value for fat, fiber, moisture, carbohydrate and energy content, which were (7.69%, 19.29%, 3.88%, 40.64% and 2629.95 k.cal), respectively. While, highest value for protein and ash contents were recorded by HC95 and HC2 which were (16.57% and 14.75%), respectively. In addition, 1.5 g/4L water m^{-2} of humic acid application showed an increase in leave contents including; protein 16.68%, fiber 20.25%, moisture 4.11%, carbohydrate 40.47% and energy 2541.52 k.cal. However, from the interaction factors V2H3 had maximum protein by almost (17.23%), and carbohydrate (40.76%). Also, maximum fat was found by (7.15%) and the energy (2708.01k.cal), with the VIH3 treatment. These facts showed that applying other doses of humic acid may cause to enhance quantity and quality of nutrient components of kenaf leaves.

KEY WORDS: Kenaf; Leaves; Humic acid; Forage; Nutrient components
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1. INTRODUCTION:

Kenaf (*Hibiscus cannabinus* L.) is a seasonal, short day, herbaceous fiber crop, belongs to the family Malvaceae (H’ng *et al*., 2009). In the world kenaf is considered as one of the important fiber crops, which consists of several parts for example, stalk, leaves and seeds with numerous valuable percentages of fibers, strands of fiber, oil, allelopathic chemicals and protein (Akinrotimi and Okocha, 2018). Lee *et al*. (2018) assumed that kenaf is a tropical plant, while nowadays kenaf can be cultivated in a broad range of geographical regions and climates. The manufacture of paper, fiber boards, bio plastic, textile and bio composites can be made from kenaf. In some established countries it’s an important cordage crop used for the production of fiber and forage, such as united states and Japan. Ryu *et al*. (2017) reported that stem of kenaf can reach to 1-2 m in height, leaves are 10-15 cm long and the flowers are 8-15 cm in diameter that can be purple, white or yellow, and the center is dark purple normally. The type of fruit is capsule and its diameter is 2 cm that comprises several seeds.

Besides of its fibers importance, leaves of kenaf contain many phenolics and antioxidants, which are used like a vegetable, especially in Asia, the components of kenaf have been regarded as vital plant-based medicines because of their therapeutic properties (Alexopoulou *et al*., 2013; Ryu *et al*., 2013). The findings presented by Sim

* **Corresponding Author:**
Deedar Maghded Sultan
E-mail: didar.sultan@su.edu.krd
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and Nyam (2021) indicated that lotion formulation prepared by 15 % kenaf seed oil with 0.1 % w/w kenaf leaves extract yields have the best physical and microbiological stability, with no toxicity on human cells. However, previous studies were also showed the impact of kenaf leaves due to a rich sources of bioactive compound such as: chlorogenic acid, caffeic aid, kaempferol, and catechin hydrate as proven (Kho *et al.*, 2019; Sim and Nyam 2019, and Haw *et al.*, 2020). Extracts of kenaf leaves and seed were non-toxic to the healthy (NIH3T3) cells, except *n*-hexane extracts, which expressed slight toxicity which was in vitro cytotoxic activity (Adnan *et al.*, 2020). The phenolic compounds detected contributed significantly to the antioxidant activities of the kenaf leaves tea. Collection kenaf leaves four months after planting was recommended for tea preparation due to it possessed high antioxidant activities (Kho *et al.*, 2019). Though, it is usually intended to the production of low economic value products: dietary fiber and animal feed (Lim *et al.*, 2020).

Kenaf is normally produced for its fiber. However, the plant, especially in silage form, can also serve as a forage crop for ruminant. Kenaf is a good source of fodder protein; the protein content is high at early growth stages, when the nutrient profile is comparable to that of alfalfa (Ryu *et al*., 2016). Optimum harvesting time for kenaf as a forage crop is at the early flowering, when plants are about six to eight weeks old. Kenaf has to be harvested at an early stage of growth, between 40-80 days after planting, to obtain high crude protein CP and optimal dry matter (DM) yield. The protein concentration in leaves decreased significantly as the harvesting date was delayed from 50 to 75 days after planting (Ammar *et al.*, 2020). Almost of seven weeks after planting is recommended to harvest *Hibiscus cannabinus* as livestock farmers and Pasture growers (Ziblim *et al.*, 2015). Additionally, the main part of the kenaf plant as a source of protein is leaf which is factitious of amino acids essential for animal growth and milk production (Noori *et al.*, 2016).

The impact of humic acid on quantity and quality of plants and also on enhancing soil was confirmed by previous studies; Shehata *et al*. (2019) studied about humic acid that is measured as a major portion of humic substance, and one of the most active materials in the soil which is consist of organic matter. Plants have been shown to be affected by these chemicals in different ways, such as morphological, physiological, genetics and biochemical influence. Kaya *et al*. (2020) informed that humic acid is a natural chemical molecule that has a physiological and biological influence on plants, as well as biological, chemical and physical impact on the soil. Humic acid has several advantages such as increased uptake of nutrients, decreasing of toxin, water retention increased, microbial growth is increased also enhanced the structure of soil. Bakry *et al.* (2014) concluded that humic acid improves the absorption of iron (Fe) and phosphorus (P), and other nutritional elements, and then recovers nutritional status of plant. It activates defense system of plant quickly; it can increase the resistance of plants to environmental stresses by stimulating growth regulators level and involved in protecting the photosynthetic apparatus and thus increasing the photosynthetic pigments and the photosynthetic machinery and thereby increasing the carbohydrate, nitrogen contents and the growth rate. Humic acid has a noticeable positive effect on the growth, yields, fiber quality and water use efficiency of salt-stressed cotton plants (Rady *et al.*, 2016).

The objective of this study was to estimate the influence of different humic acid concentrations on nutrient components of leaves of different kenaf varieties.

2. MATERIALS AND METHODS

2.1. Martials

Three varieties of kenaf (*Hibiscus cannabinus* L.) as the plant material were used as shown in (Table 1).

Humic acid was used in the rates of (0.0, 0.5, 1.0, 1.5 g/4 L water m^{-2}), were normal tap water used, (BioHumic, 95% humic acid, 100% Soluble, USA, Produced by: Plant’s Choice; Batch number: 201211). In addition, NPK, 13:2:44 fertilizer must be added as basal application in the rate of (150 kg ha^{-1}), produced by the SQM Company.

Table 1. Kenaf varieties were used in the studied project.

Variety	Country of origin
FH952	Fujian Fuzhou, China

HC95	Bangladesh
HC2	Bangladesh

The Institute of Tropical Forestry and Forest Production (INTROP) at the Universiti Putra Malaysia provided seeds of all kenaf varieties.

2.2. Study Site

The experiment was conducted at two locations, first at Grdarasha Field, College of Agricultural Engineering Sciences, Salahaddin University-Erbil, which is located at (Latitude 36° 00 16 N and Longitude 44° 01 24 E), and elevation of 398 meters above sea level. Second at Ainkawa Research Station, which was located at (Latitude 36° 14 32 N, and Longitude 43° 59 33 E), and elevation of 415 meters above sea level. Figure 1 display the geographical location of the study sites.

2.3. Soil Sampling

The soil samples were taken randomly at the depth of 0 to 15 cm from several places on the land before it was divided into plots. Then, the samples transported to the laboratory. Afterward, the soil was air dried and sieved through a 2 mm pore size sieve. The physical and chemical properties of the soil at both locations were shown in Table 2.

Table 2. The physical and chemical properties of the soil of Grdarasha and Ainkawa research station.

Soil properties		**Grdarasha**	**Ainkawa**
Physical properties	**Sand %**	31	19
	Silt %	37.3	43
	Clay %	31.7	38
	Soil texture	Clay loam	Silty clay loam
Chemical properties			
	EC(Ds/m)	0.5	0.3
	PH	7.83	7.86
	N %	0.07	0.11
	P(PPM)	12.5	8.62
	K(PPM)	338	200
	O.M %	1.14	0.9

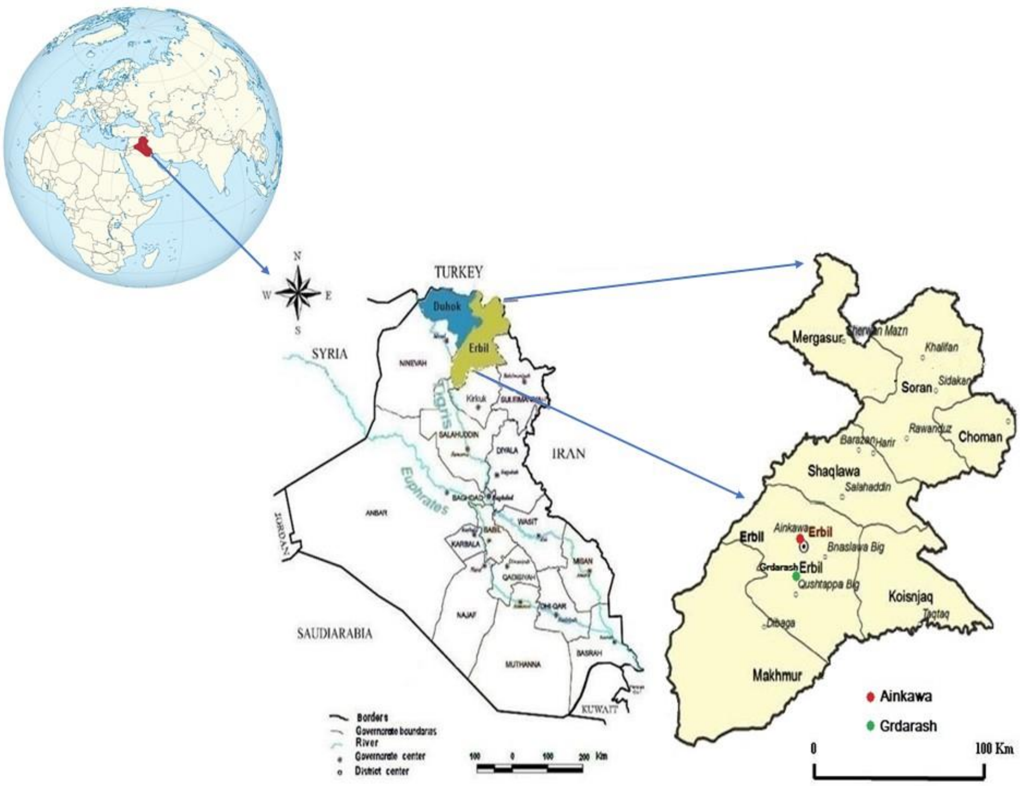

Figure 1. Geographical location of the study sites.

2.4. Experimental Design

The experiment was carried out in two locations, the first location at Gerdarasha Field in College of Agricultural Engineering Sciences, Salahaddin University-Erbil, and the second location was in Ainkawa Research Station, Erbil, during summer season in the middle of April and early of May 2021. Factorial Randomized Complete Block Design (RCBD) with three replications were practiced. The first factor consist of three kenaf varieties were used such as FH952, HC95 and HC2 as (V1, V2 and V3), respectively. While, the second factor was humic acid which applied to the plants by four different concentrations 0 as a control treatment with (0.5, 1.0, 1.5 g/4L water m^{-2}), which were symbolled by (H0, H1, H2 and H3), respectively. Three seeds were placed in each hole at the depth of 1-2 cm. Each plot in all replications was 1 m^{-2}, the space between plants was 10 cm, and 33 cm apart between the rows, while 1 m between plots with 1.5 m between replications. After NPK fertilizer application humic acid was added two weeks later.

2.5. Data collection

From each treatment five kenaf plants were selected randomly, then nine leaves were chosen in bottom, middle and top an around of the stems in each plant. Next, the leaves were removed and transported to the laboratory for oven drying at 70°C for 48 hours. To determine the percentage of chemical components; protein, fat, ash, fiber, moisture and carbohydrate, and also energy of leaves. So, for detecting each component the following equations were used:

$$\text{Crude protein} = \frac{1.4 \times sulfuric\ acid\ 0.1\ N \times \text{nitrogen rate} \times \text{titration rate of nitrogen}}{sample\ weight\ (2\ g)} \times 100$$

$$\text{Fat} = \frac{flask\ weight - (\text{sample weight} + flask\ weight + 100\ ml\ ethar)}{sample\ weight\ (2\ g)} \times 100$$

$$\text{Ash} = \frac{tube\ weight - (\text{sample weight} + tube\ weight)}{sample\ weight\ (2\ g)} \times 100$$

$$\text{Fiber} = \frac{beaker\ weight - (\text{sample weight} + beakar\ weight + 12.5\ ml\ H2SO4)}{sample\ weight\ (2\ g)} \times 100$$

$$\text{Carbohydrate} = (crude\ protein + fat + ash + moisture + fiber - 100\ )$$

$$\text{Energy} = (crude\ protein \times 3.5 + fat \times 8.5\ + \text{Carbohydrate} \times 3.5\ ) \times 10$$

2.6. Statistical Analysis

Chemical components of leaves were statistically analyzed according to the technique of analysis of variance (ANOVA) for randomized complete block design, (RCBD) using IBM SPSS Statistics program (20) the mean comparison was fulfilled according to Duncans 1955 range test at the level of significant 0.05.

3. RESULTS

3.1. Effect of humic acid and varieties on leave contents

Table 3 showed the main compression of leave contents including protein %, fat %, ash %, fiber %, moisture %, carbohydrate % and energy k.cal. at both locations. The results indicated that all evaluated leave contents as mentioned before significantly influenced by using different varieties, humic acid and their interactions at Grdarasha and Ainkawa fields.

3.2. Effect of varieties and Humic Acid Application on Protein Content %

Protein is one of the important contents in kenaf leaves that increased by humic acid application. The results of Grdarasha field showed that highest and lowest protein content were 16.57 % and 15.63 % as recorded by (V2 and V1), respectively. In addition, 1.5 g/4L water m^{-2} of humic acid submission recorded highest protein content was 16.68 % followed by 16.36 % when humic acid added at 1.0 g/4L water m^{-2}. The interaction of factors V2H3 and V1H3 had 17.23 % and 15.23 % as maximum and minimum for protein content.

As well as, at Ainkawa field V2 and V1 had 17.86 % and 17.08 % protein content as highest and lowest value. While, 17.92 % of protein were recorded by control treatment followed by 17.38 % when humic acid was added by 1.0 g/4L water m^{-2}. Additionally, in Ainkawa field was also the interaction factors significantly affected protein content as found in these treatments; V2H1 and V1H2 which values almost (18.74 % and 16.65%), respectively as showed in (Figure 2).

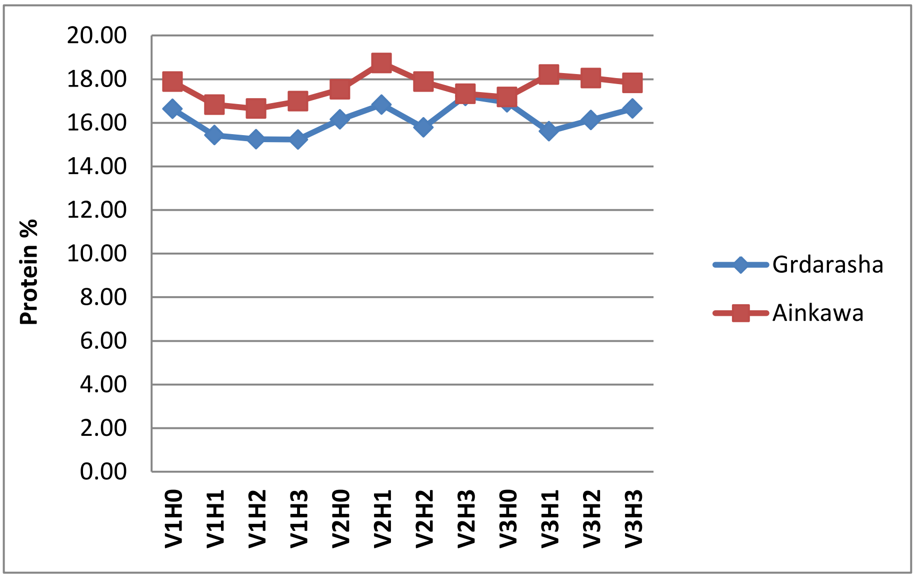

Figure 2. Protein content % of kenaf leaves at Grdarasha and Ainkawa fields.

3.3. Effect of Varieties and Humic Acid Application on Fat Content %

According to table 3 the fat content was significantly affected by using different varieties and levels of humic acid and their interactions at both fields. At Grdarasha field V1 recorded highest fat content 7.69 % followed by V3 and V2 were 6.91 % and 6.44 % respectively. While, 0.5 g/4L water m^{-2} of humic acid was sufficient for increasing fat content to 7.70 % as compared to control treatment. In the interactions of factors clearly can be seen that V1H2 had 8.10 % fat, whereas V2H1 had 4.64 % fat in there leaves. Also, at Ainkawa field again V1 and V2 had 6.71 % and 5.63 % of fat as maximum and minimum value respectively. However, fat content not increased by humic acid application, hence highest value recorded by control treatment 6.15 %. For interaction of factors V1H3 had 7.13 % fat while V2H3 had 4.58 % of fat as showed in (Figure 3).

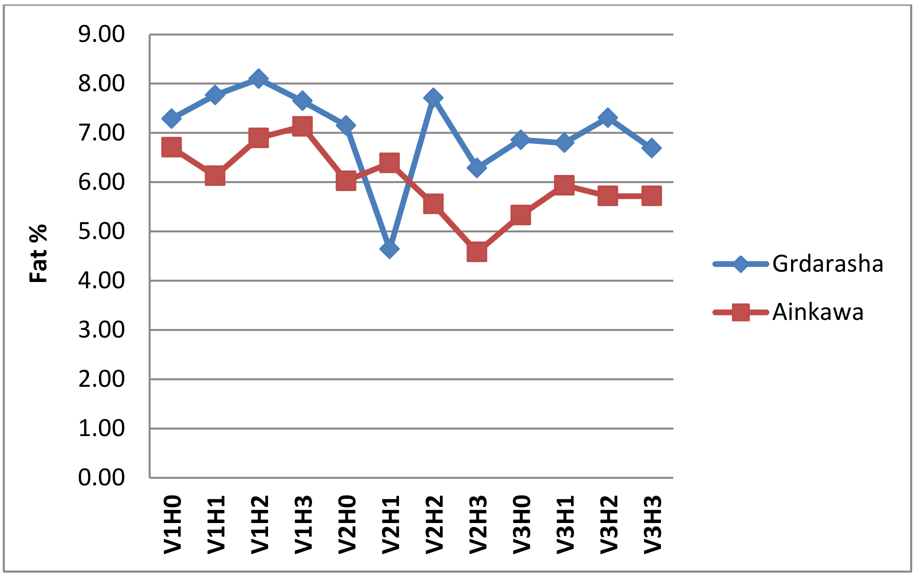

Figure 3. Fat content % of kenaf leaves at Grdarasha and Ainkawa fields.

3.4. Effect of Varieties and Humic Acid Application on Ash Content %

Ash content significantly impacted by variety, humic acid and their interactions at Grdarasha field (Table 3). There for, V3 recorded maximum ash content 14.75 % and V1 recorded minimum ash content 12.81 %. For increasing ash content of kenaf leaves jest 0.5 g/4L water m^{-2} of humic acid is needed were recorded 14.32 % of ash as compared to other treatments. In the interaction of factors maximum and minimum ash content were 15.42% and 11.87 % with V3H0 and V1H3 respectively. At Ainkawa field as well V3 had highest ash content 11.94 % followed by 11.23 % for V1 as lowest value. Whereas, at Ainkawa field more humic acid required for increasing ash content were 1.0 g/4L water m^{-2} had 12.04 % ash as compared to other treatments. On the other hand, V1H0 and V2H0 recorded 12.53 % and 10.53 % of ash respectively as showed in (Figure 4).

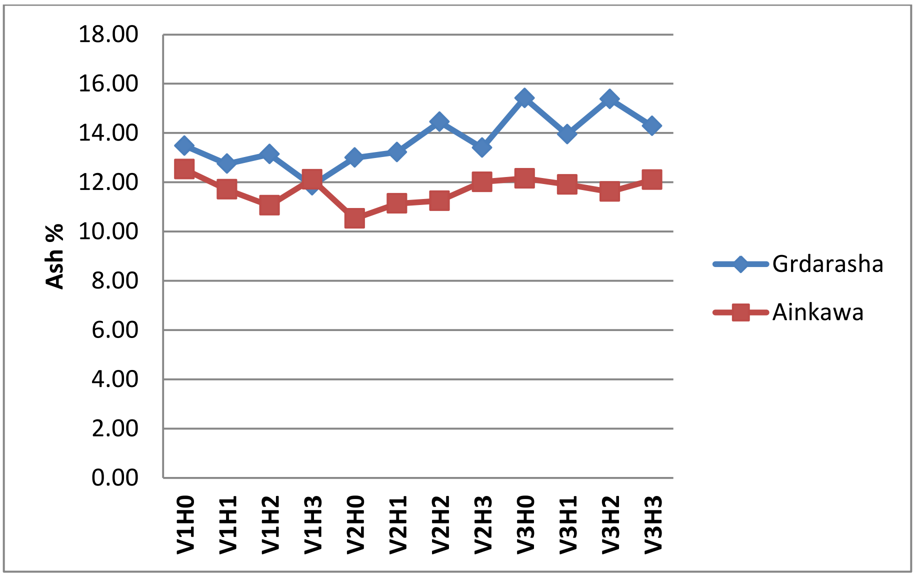

Figure 4. Ash content % of kenaf leaves at Grdarasha and Ainkawa fields.

3.5. Effect of Varieties and Humic Acid Application on Fiber Content %

Fiber percentage of kenaf leaves at Grdarasha field significantly affected with different varieties, humic acid and their interactions (Table 3). Therefore, the results represented that maximum fiber % were 19.29 % for V1 as compared to other varieties, and 1.5 g/4L water m^{-2} of humic acid application showed an increase in fiber content which was 20.25 % as compared to control treatment. While, as showed in figure 5 fiber % for V3H0 were 20.51 %. At Ainkawa field as well V1 recorded 21.53 % as compared to V3 which were 19.64 %. On the other hand, V2H2 had highest value 22.32 % as showed in (Figure 5).

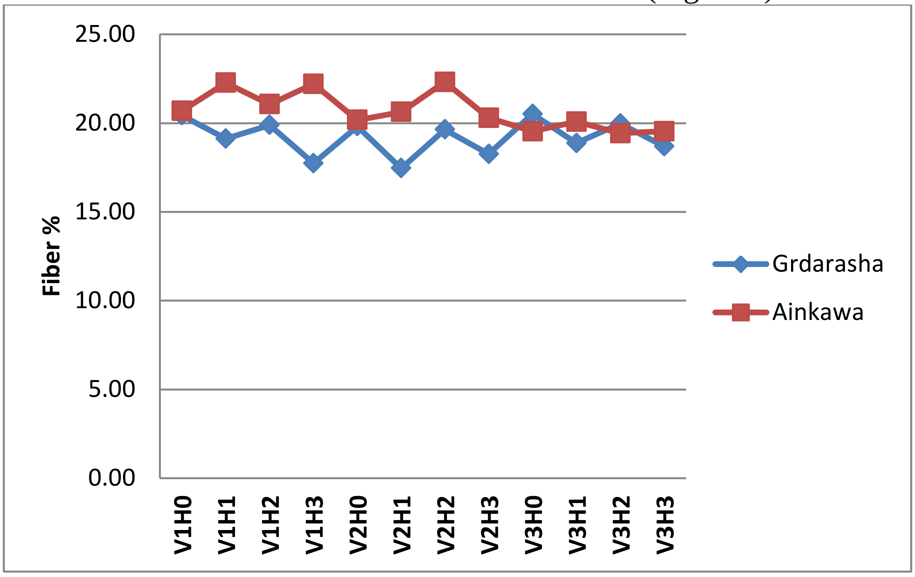

Figure 5. Fiber content % of kenaf leaves at Grdarasha and Ainkawa fields.

3.6. Effect of Varieties and Humic Acid Application on Moisture Content %

Table 3 and figure 6 show the significance differences between factors were applied from this current study on the moisture content. Results of grdarasha field showed that maximum and minimum moisture content were 4.12 % and 3.92 % for V2 and V1 respectively. Moisture content increased by 1.0 g/4L water m^{-2} of humic acid application to 4.15% as compared to control treatment 3.90 %. The interaction of factors determined that V2H3 and V1H1 recorded highest and lowest moisture content which were (4.40 % and 3.92 %), respectively. However, the results at Ainkawa field showed that V1 and V3 had highest 3.88 % moisture, while V2 had lowest moisture 3.81%. Maximum and minimum moisture were 4.11 % and 3.64% for 1.5 and 1.0 g/4L water m^{-2} respectively of humic acid application. High moisture content was found in the treatment V2H0 by 4.21% in compassion to other treatments.

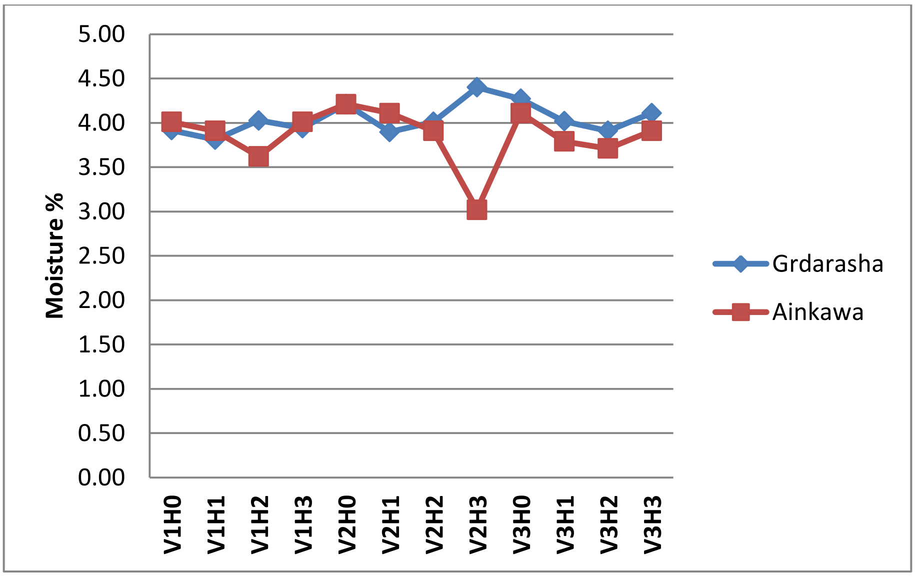

Figure 6. Moisture content % of kenaf leaves at Grdarasha and Ainkawa fields.

3.7. Effect of Varieties and Humic Acid Application on Carbohydrate Content %

Carbohydrate is an important ingredient in leave composition that used for animal feed, so the results of grdarasha field displayed that V1 and V3 recorded 40.64% and 38.43% of carbohydrate respectively. According to figure 7 maximum and minimum value for carbohydrate were 43.96 % and 36.03 % for V2H1 and V3H0 respectively. In contrary, in Ainkawa field the results were adverse for varieties in which V3 had 41.04 % and V1 had 38.84 % carbohydrate. When 1.5 g/4L water m^{-2} of humic acid applied carbohydrate content increased to 40.47 % as compared to control which were 39.42 %. In addition, V2H3 had 42.77 % as highest carbohydrate content, while lowest were 37.63% for V1H3.

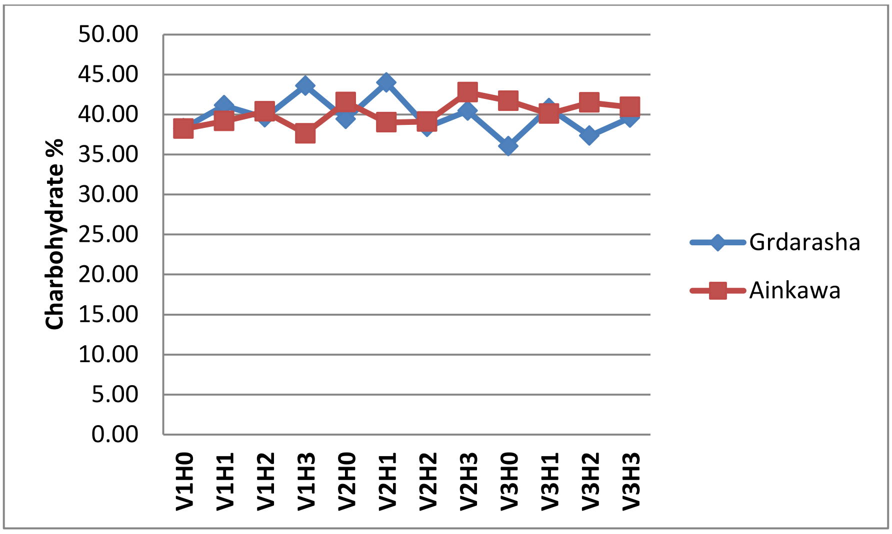

Figure 7. Carbohydrate content % of kenaf leaves at Grdarasha and Ainkawa fields.

3.8. Effect of Varieties and Humic Acid Application on Energy Content %

This parameter were taken before flowering stage, thus results of Grdarasha field indicated there were significant differences between varieties (Table 3), in which maximum and minimum energy were (2629.95 and 2503.60 k.cal) for V1 and V3 respectively. For the interaction of factors were V1H3 had highest energy of 2708.02 k.cal, while V3H0 had lowest energy of 2435.91 k.cal (Figure 8). Although, the highest value was by 2542.12 k.cal for V3 and for V2 was by 2525.28 k.cal at Ainkawa. 1.5 g/4L water m^{-2} of humic acid application were sufficient for increasing energy to 2541.52 k.cal. Highest and lowest energy were by (2588.43 and 2465.98 k.cal) for V1H2 and V2H2 respectively.

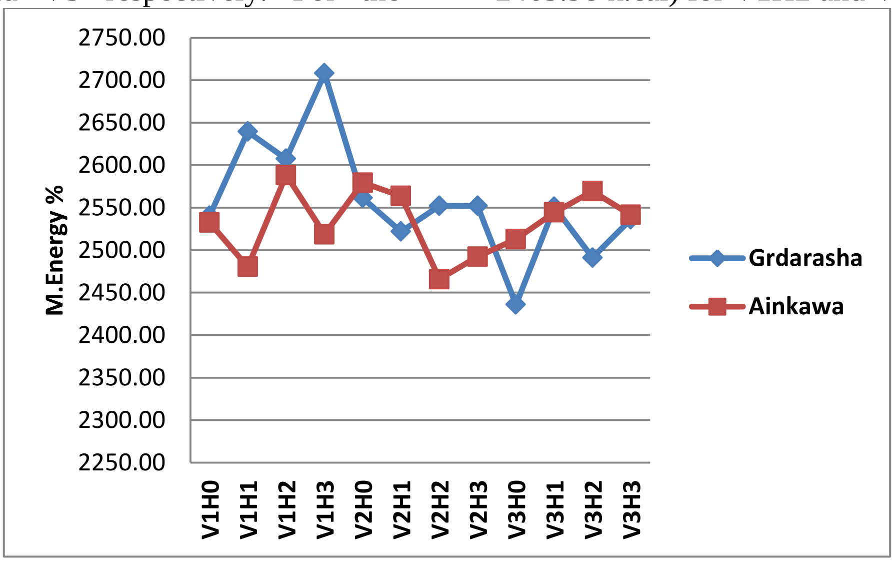

Figure 8. M. Energy k.cal. of kenaf leaves at Grdarasha and Ainkawa fields.

Table 3. The analysis of variance (ANOVA) for the influence of leave contents and their interactions at Grdarasha and Ainkawa fields.

		Protein%		Fat%		Ash%		Fiber%		Moisture%		Carbohydrate%		Energy k.cal.	
Grdarasha															
Source	**DF**	**MS**	**F.V**	**MS**	**F.V**	**MS**	**F.V**	**MS**	**F.V**	**MS**	**F.V**	**MS**	**F.V**	**MS**	**F.V***
V	2	2.8	3866.1*	4.8	15600.8*	11.6	122953.7*	1.6	5890.5*	0.13	950.3*	18.8	2320.6*	49460.5	340.6*
H	3	1.6	2222.0*	2.6	8552.8*	2.6	27946.7*	8.8	32321.3*	0.12	859.6*	36.1	4446.6*	11604.4	79.9*
V×H	6	0.9	1254.0*	1.6	5353.9*	0.8	9299.7*	0.6	2411.9*	0.05	407.3*	5.4	667.6*	5759.1	39.6*
Ainkawa															
V	2	2.2	3826.5*	4.5	18310.4*	1.8	7113.1*	11.0	42281.2*	0.02	204.7*	16.3	1129.4*	906.6	109.0*
H	3	0.4	818.2*	0.1	729.5*	0.9	3478.7*	1.4	5524.3*	0.38	3802.0*	2.2	154.7*	1178.3	141.7*
V×H	6	1.0	1819.3*	1.2	4886.1*	0.7	3191.1*	1.9	7303.6*	0.34	3483.7*	6.9	482.2*	7713.8	927.8*

* Significant at 5%, when p-value less than 0.05 (typically ≤ 0.05).
**V = Variety, H = Humic acid, V×H = Interaction treatment, DF= Degrees of freedom, MS= Mean square, F.V= F. value

4. DISCUSSION

Chemical contents of kenaf leaves are affected by varieties and humic acid as applied from this current study. Protein content significantly enhanced by both factors (varieties and humic acid), similar result in the same plant but in the different part was found by Salih *et al.* (2014) who indicated that the protein content of the kenaf fibers were significantly influenced by variety and fertilizers. Additionally, Kipriotis *et al.* (2007) reported that nitrogen rates in stems and leaves were influenced by using different fertilizers and varieties. Total nutrient content substantially different among varieties of kenaf plant. Highest and lowest total nutrient content gave by HC2 and HC95 were (45.40 and 37.51 g plant^{-1}), respectively (Hossain *et al.,* 2011). The maximum protein and iron concentration was found in 2 % urea, and lowest in control (Omenna and Oduwaye, 2017). Hossain *et al.* (2011) declared that the variety HC2 had the maximum quantity of nitrogen in leaves 42.15 g kg^{-1} and potassium in stem 11.24 g kg-1. Due to this variation in nutrient concentration of different kenaf plant parts, nutrients were partitioned according to plant sections compared to the total quantity of nutrients measured in the entire plant. A change in the content of one nutrient in a balanced system may increase or decrease the concentration of other nutrients. So, the chemical components were influenced not just by organic or inorganic fertilizers but also by growth stages as Basri *et al.* (2014) reported that crude protein content was 30 % which was at 28 days after planting while it decreased to 20 % at 56 days after planting. Kenaf plant cell wall and total content of carbohydrate decreased after flowering, followed by a modest rise at maturity (Ziblim *et al.,* 2015). Ammar *et al.* (2020) concluded that when kenaf is utilized in any type of roughage, aspects for example growing stage or plant component impacting nutritional content should be carefully studied. Leaves of kenaf contain more crude protein and less fiber as compared to stalk and are more digestible, while the variations between leaves and stems get larger as the plant matures. Jo and Byamungu (2020) in an investigation used organic and other fertilizer, and the results showed that 250 kg ha^{-1} of N increased yield of dry matter. As well as, leaves crude protein content at 200 and 250 kg ha^{-1} of N application was the same and were greater as compared to other types of fertilizers. The organic fertilizer application rates of 250 kg ha^{-1} of N resulted in greatest crude protein concentration in the stem 4.3 %. There was no significance difference of fiber in leaves between chemical and organic fertilizer application. Hossain *et al.* (2014) who used carbon fertilizer in different rates, and results indicated that nitrogen percentage in leaves were maximum at 20 t ha^{-1} as compared to control treatment. However, after 10 t ha^{-1} of carbon the nitrogen in roots and stems declined while calcium, potassium, magnesium and phosphorus in leaves were increased at 20 t ha^{-1}. HC2 variety showed highest value of NPK in leaves and total nutrient content. Paksoy *et al.* (2010) concluded that humic acid not only improved macro-nutrient contents, but also increased micro-nutrient contents of the plant organs. Moreover, humic acid application and plant inoculation with *Glomus intraradices* increased vitamin C, anthocyanin, and phenolic compounds in *Hibiscus sabdariffa* L (Fallahi *et al.,* 2017). This current study was also strongly supported by similar results were confirmed that humic acid (HA) can improve the growth, yield, nutrient uptake, chlorophyll content, carotenoids, and leaf carbohydrates in roselle plant (Sanjari *et al.,* 2015).

5. CONCLUSIONS

Generally, the results of nutrient content in leaves of keanf plant showed that the superiority goes to FH952 variety due to an increase in fat, fiber, moisture, carbohydrate and energy. Despite that, the biggest value of protein content was found by Bangladesh variety HC95. Moreover, adding humic acid increased the leaves nutrient content as compared to control treatment. This approves utilization of humic acid enhances nutritional value of leaves be useful as forage for animal feed, since these components are the stander should be have in any forage crops. However, due to the findings from this study which recommend that applying other levels and types of humic acid may improve nutritive value of keanf leaves has impact not just for livestock but also for human uses as food, cosmetic and medicine.

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Conflict of Interest

The authors declare no conflict of interest

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