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PHYSICOCHEMICAL COMPOSITION OF KENAF(*Hibiscus cannabinus L.*) SEED UNDER DIFFERENT STORAGE TEMPARATURE.

COMPOSICIÓN FÍSICOQUÍMICA DE SEMILLAS KENAF (*Hibiscus cannabinus* L.) BAJO DIFERENTES TEMPERATURAS DE ALMACENAMIENTO.

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ABSTRACT

Kenaf is an annual plant grown for its fibre which basically depend on the seed viability, for its maximum growth and production.The seed viability and vigor in turn depend on storage condition. There is insufficient information on physicochemical composition of kenaf seed in relation to seed viability. The present study was performed to know the physicochemical composition of the kenaf seed at different storage temperature in other to improve upon rapid loss of seed viability in kenaf. Four varieties of kenaf seed were obtained from I.A.R. & T seed unit . The seeds were stored at 0^0C(Unviable seed), 2.2^0C(Lower temperature), 35^0C(Ambient temperature), 28^0C(Cold room temperature which serve as control) for period of 8 months. Seed germination test and weight was performed before and after the experiment. Proximate analysis (Crude protein, Moisture, Carbohydrate, Ash, Crude fibre, Crude fat) and Phytochemicals (Total phenolics, Steroid and Total Flavonoids were also determined). The result shows that there is significant difference in all the parameters studied, for all the storage temperatures as compared with the control (28^0C) among the varieties except from ifeken 100 where the was no significant difference in Crude fibre and Ash content for seed under ambient temperature as compared with the control. However, the level of tolerance to different storage temperature among the varieties following the order Ifeken 400 >Tianung 2 > Cuba 108>Ifeken 100.

Keywords: Kenaf, Storage, Seed germination, Seed weight, Temparature, Seed viability, Proximate, Flavonoid ,Phenolics.

RESUMEN

Kenafesunaplantaanualcultivadaporsufibraquedependebásicamente de la viabilidad de la semilla, parasumáximocrecimiento y producción. La viabilidad y el vigor de la semilla a suvezdependen de lascondiciones de almacenamiento. El presenteestudio se realizóparaconocer la composiciónquímica de la semilla de kenaf a diferentestemperaturas de almacenamiento.Se obtuvieroncuatrovariedades de semillas de kenaf de I.A.R. & T unidad de semillas. Las semillas se almacenaron a 00 ° C (semilla no viable), 2,20 ° C (temperaturamásbaja), 350 ° C (temperaturaambiente), 280 ° C (temperaturaambientefríaquesirvecomo control) durante un período de 8 meses. La prueba de germinación de semillas y el peso se realizaron antes y despuésdelexperimento. Análisispróximo (proteínacruda, humedad, carbohidratos, cenizas, fibracruda, grasacruda) y fitoquímicos (también se determinaronfenólicostotales, esteroides y flavonoidestotales). El resultadomuestraqueexisteunadiferenciasignificativaentodos los parámetrosestudiados, paratodaslastemperaturas de almacenamientoencomparación con el control (280C) entre lasvariedades, excepto de ifeken 100, donde no hubounadiferenciasignificativaen el contenido de fibracruda y cenizasparasemillasbajoambiente. temperaturaencomparación con el control. Sin embargo, el nivel de tolerancia a diferentestemperaturas de almacenamiento entre lasvariedadessiguiendo el ordenIfeken 400>Tianung 2> Cuba 108>Ifeken 100.

Palabras clave: Kenaf, almacenamiento, germinación de semillas, peso de semillas, temperatura, viabilidad de semillas, proximal, flavonoide, fenólicos.

INTRODUCTION

Kenaf is an industrial fibre crop belonging to Malvaceae family which is closely related to okra and cotton. The seed is small (1.5-3.3g/100 seeds), black in color and subreniform in shape. In terms of storage, the maximum seed moisture content for bagging seed under controlled temperature and humidity is 16.5%, seed moisture content is preferably 14% or less (Scott and Cook, 1995). In the past,chemical seed treatment is a common agriculture practice to protect crop seed viability and prevent reduction from pathogens. However, no chemical seed treatment is presently registered for use on kenaf seed, research has demonstrated the safety and benefits for kenaf seed (Cook et al., 1992), Normally,under ordinary storage conditions the seed retains its viability for about 8 months. Kenaf seeds contains large amount of oil. Kenaf seed oil is used for cosmetics, industrial lubricants and for biofuel production. The oil is high in omega polyunsaturated fatty acids (PUFAs) and contain

high percentage of linoleic acid (Omega-6). Adebisi et al.(2013) had reported that kenaf seed remains viable for about 6 months at ambient storage condition but, due to the fact that Kenaf seed has high oil content averaging 23.7% , care should be taken to preserve the seed viability, especially when proposing long-term seed storage. Commonly to other crop seeds with high oil percentages, seed viability decreases over time when stored at higher relative humidity (RH) and higher temperatures(Mohamed et al.,1995) . In addition, Charles and Venita 2002 had reported that Toole et al., 1960 had found out that Research on kenaf seed storage indicated that seed stored at 8% RH remained fully viable for 5.5 years when stored at either –10°, 0°, or 10°C, and fully viable for 5.5 years when stored at –10° or 0°C at 12% RH . The high oil content ofkenaf makes it to be vulnerable to seed deterioration if it is not well stored. Ali et al. (1995) have reported chemical composition of kenaf seed oil. Adebisi et al., 2013 have reported shelf life of kenaf stored under humid tropical condition. He reported that Ramamothy, 1989 had found out that even though kenaf is in good storage, the signs of physiological deterioration in terms of slower germination and reducing seedling growth of carry over seeds are apparent. Therefore, there is need to determine physicochemical composition of kenaf seed under different storage condition hence this study on physiochemical composition of kenaf (Hibiscus cannabinus L.) seed under different storage temparature

MATERIALS AND METHODS

Plant materials: Four varieties of kenaf seed (Ifeken 400, Ifeken 100, Tianung 2, and Cuba 108) were obtained from seed unit of Institute of Agricultural Research and Training (I.A.R. & T) Ibadan.

Methods: Seed germination test were performed on the cleaned and sorted seed varieties to ascertain the seed viability before the experiments. Seeds were later subjected to different storage temperature (lower temperature (2.2^0C), extreme temperature (50^0C) and ambient temperature(35^oC),unviable seed(0^oC) while cold room temperature(28^oC) serves as control. The seed were stored for 8 months after which proximate and phytochemical analysis, seed germination test, seed weight were determined. The experiment were laid out in randomized block design with three replicates per treatment.

Seed germination test and Seed weight: Seed germination test and Seed weight were determined according to the modified method of (Sigh and Gosh 2010)

Seed germination test: Twenty seeds were planted on filter paper with 5ml of distilled water added inside the petridishes and were allowed to germinate for 7 days after which the percentage germination(%) were recorded and calculated as:

Number of seed germinated/Total number of seed X 100

Seed weight: Twenty seeds were placed in a petridish and weighed on analytical balance

Moisture Content: Moisture content determination was carried out using the air oven method according to the A. O. A. C (2005). Calculation:

% Moisture content = Weight Loss/Weight of sampleX 100

Ash content: Ash content was determined using muffle furnace according to the method A. O. A. C (2005).

Percentage Ash = Weight loss / Weight of sampleX 100

Crude fat: The soxhlets extraction method (AOAC, 2002) was used to determine the crude fat. The fat extracted from a given quantity of sample was then calculated:

% Fat (W/W) =Loss in Weight of sample / Original Weight of SampleX 100

Crude Protein: The crude protein content was determined using micro Kjeldahl method as described in AOAC (2005).

%N = (Molarity of HC1 X Sample titre – Blank titre) X 0.014 X DF) / Weight of sample usedX 100.

% N was converted to the percentage crude protein by multiplying by 6.25.

Crude Fibre: The residue obtain from fat extraction was used to determine crude fibre after series of digestion and washing ,it was then was cooled and weighed. The loss on ignition was reported as crude Fibre (AOAC, 2005).

Carbohydrate: The carbohydrate content was calculated by difference.

% CHO = 100-(Sum of the percentages of moisture, ash, fat, protein and crude fibre)

Total Flavonoids: Total flavonoid was determined according to the method of Hung and Nhi(2012).1g of seed was extracted with 10ml of 80% Methanol. Total Flavonoids content was calculated as mg/g Quercetin form calibration graph

Total Phenolics: Total Phenolics was determined according to the method of Hung and Nhi(2012) 2g of seed was extracted with 20ml of 80:20 Acetone:0.5% formic Acid . Total Phenolic content was calculated as GAE/g form calibration n graph

Steroid: Steroid was determined according to method of Wall *et al.,*1952. 0.50g of sample extract was weighed into a 100ml beaker.20ml of Chloroform-Methanol(2:1).Standard Steroids of concentration of 0-4mg/ml were prepared from 100mg/ml stock steroid solution ande treated similarly like sample as above. % Steroid was calculated using the formula:

(Absorbance of Sample X Gradient X Dilution Factor)/(Wt of sample X 10000)

Statistical analysis: Data were analysed using IBM (SPSS) 23. Means were compared using Anova, and were separated using Duncan multiple range test at $p<0.05$ level of significance.

RESULTS AND DISCUSSION

Effect of storage temperature on Seed germination and seed weight among kenaf varieties, the result in Table 5,6,7 and 8 shows that there is significant difference in the seed germination and seed weight for all the storage temperature as compared to the control which indicate the deleterious effects of the unsuitable storage condition on the seed as growth and development of kenaf solely depend on seed germination and vigor . This was also in accordance with the work of Adebisi*et al* 2013 who reported a more favourable storage temperature of 27.5^{o}C and 64.68% Relative humidity for higher Seed viability and Seedling vigour index. AlsoWebber III and Bledsoe, 2002 also reported that seed viability decreases over time when stored at higher temparature.

Effects of Storage Temparature on Proximate Composition among Kenaf varieties: Table 1,2,3 and 4 shows that there is significant difference in the level of crude protein, moisture, carbohydrate, fat, ash and crude fibre for all the storage temperature examined among the kenaf varieties except for Ash and crude fibre in Ifeken 100 for (35^0C) which show no significant difference as compared with control. This was in according to findings of Webber III and Bledsoe 2002 that moisture content affect nutritional composition and yield of Kenaf. They also reported that Kenaf industry report plant yield in an oven-dry basis at 0% moisture.

Table 1. Effect of temperatures on Proximate composition of kenaf seed (Ifeken 100)
Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature ^{0}C	Crudeprotein(%)	Moisture (%)	Carbohydrate(%)	Fat (%)	Ash(%)	Crude fibre(%)
KS (0.0)	20.93 ± 0.23*	7.27± 0.09d*	33.44 ± 0.12*	12.08±0.06*	5.27± 0.09*	21.01±0.12*
KS (2.2)	20.17 ± 0.27*	12.57± 0.12*	29.28 ± 0.12*	12.10±0.06*	4.85± 0.06*	21.03±0.27*
Control (28.0)	23.87 ± 0.09	8.93± 0.09	24.20 ± 0.10	14.67±0.09	5.83± 0.09	22.50±0.31
KS (35.0)	23.30 ± 0.06*	8.13± 0.09*	26.23 ± 0.12	14.10±0.06*	5.81±0.03ns	22.43±0.69ns
KS (50.0)	20.57 ± 0.09*	6.23± 0.09*	34.78 ± 0.15*	12.02±0.06*	5.23±0.09*	21.17±0.09*

Table 2.Effect of temperatures on proximate composition of kenaf varieties (Ifeken 400).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature ^{0}C	Crude protein (%)	Moisture (%)	Carbohydrate(%)	Fat (%)	Ash(%)	Crude fibre(%)
KS (0.0)	22.57 ± 0.09*	7.50± 0.06*	28.86 ± 0.06*	13.97±0.09*	5.40± 0.06*	21.70±0.12*
KS (2.2)	22.63 ± 0.27*	10.20± 0.06*	25.43 ± 0.15*	14.17±0.09*	5.60± 0.06*	21.97±0.09*
Control (28.0)	24.30 ± 0.36	9.03± 0.09	21.41 ± 0.52	15.33±0.12	6.13± 0.09	23.80±0.06
KS (35.0)	23.47 ± 0.27*	8.37± 0.03*	24.73 ± 0.23*	14.67±0.09*	5.99± 0.06*	22.77±0.09*
KS (50.0)	21.77 ± 1.02*	6.87± 0.09*	30.82 ± 0.12*	13.53±0.09*	5.38±0.06 *	21.63±0.09*

Table 3. Effect of temperatures on proximate composition of kenaf varieties (Cuba 108).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature ^{0}C	Crude protein (%)	Moisture (%)	Carbohydrate(%)	Fat (%)	Ash (%)	Crude fibre(%)
KS (0.0)	21.10 ± 0.06*	6.07± 0.09*	35.40 ± 0.15*	12.03±0.09*	4.53± 0.06*	20.87±0.12*
KS (2.2)	20.89 ± 0.09*	10.70± 0.06*	29.29 ± 0.15*	12.87±0.09*	5.02± 0.06*	21.23±0.09*
Control (28.0)	22.63 ± 0.09	8.50± 0.12	26.04 ± 0.39	14.83±0.12	5.67± 0.09	22.33±0.24
KS (35.0)	21.93 ± 0.24*	7.63± 0.09*	29.42 ± 0.06*	13.69±0.09*	5.33± 0.09*	22.00±0.12*
KS (50.0)	20.73 ± 0.09*	5.87± 0.09*	35.77 ± 0.09*	12.13±0.09*	4.77±0.09*	20.73±0.12*

Table 4. Effect of temperatures on proximate composition of kenaf varieties (Tianung 2).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature ^{0}C	Crude protein (%)	Moisture (%)	Carbohydrate(%)	Fat (%)	Ash(%)	Crude fibre(%)
KS (0.0)	21.17 ± 0.19*	6.10± 0.06*	34.93 ± 0.09*	12.07±0.09*	4.70± 0.06*	21.03±0.15*
KS (2.2)	21.43 ± 0.09*	9.43± 0.09*	30.52 ± 0.09*	12.16±0.09*	5.09± 0.06*	21.37±0.09*
Control (28.0)	23.23 ± 0.09	8.80± 0.06	23.97 ± 0.40	14.93±0.09	5.90± 0.06	23.17±0.09
KS (35.0)	22.13 ± 0.09*	7.69± 0.09*	28.75 ± 0.09*	13.60±0.17*	5.70± 0.06*	22.13±0.09*
KS (50.0)	21.13 ± 0.09*	6.30± 0.06*	34.31 ± 0.06*	12.17±0.09*	4.89±0.06*	21.20±0.06*

Effects of Storage Temparature on Phytochemicals among Kenaf varieties: Fig 1-12shows that there is significant difference in the Total Phenolics , flavonoids and steroid for all the storage temperature as compared with the control among the kenaf varieties. Flavonoids and phenolic compounds are present in several plants and serve to inhibit lipid peroxidation and lipoxygenases.Lipoxygenases are non-heme iron enzymes that catalyze the dioxygenation of polyunsaturated fatty acids (Linoleic acid) to yield hydroperoxides (Taner*et al.,*2006). Although, Montanaro and Dichio2012 reported that (Jaakola*et al.*, and Mika et al., 2004), find out that Flavonoids are organic molecules that have been shown to have a protective role against several stresses both by themselves and in conjugation with peroxidases. . Steroid are of different types in plant and play diverse role but majorly acting as signaling molecules. The activity of these phytochemicals was significantly reduce due to deterioration of the seed resulting from unfavourable storage temparatures. This is because rate of synthesis of reactive oxygen species generated from unfavourable temperature surpasses the antioxidant potential of this phytochemicals.This was also in accordance with the work of Tiwari et al.,2013 who reported that Hager et al 2008 found out that there is dramatic losses in total anthocyanin follow heating and storage of frozen black raspberries for six months.

However, the significant difference observed among the kenaf varieties was as a result of the deterioration of the nutrient composition of seed due to exposure to unfavourable storage temperature. Therefore, the level of tolerance to different storage temperatures among the varieties shows in their respective physical and chemical compositions which follow the order of Ifeken 400 >Tianung 2 > Cuba 108 >Ifeken 100 for period of 8 months. This was also in accordance with the finding of Adebisi*et al.,* 2013 who reported that the highest seed longetivity of kenaf seed is 8-9 months. Moreover, the

difference in values among kenaf varieties was as a result of their differences in their genotype which majorly contributed to their responses to different storage temperatures.

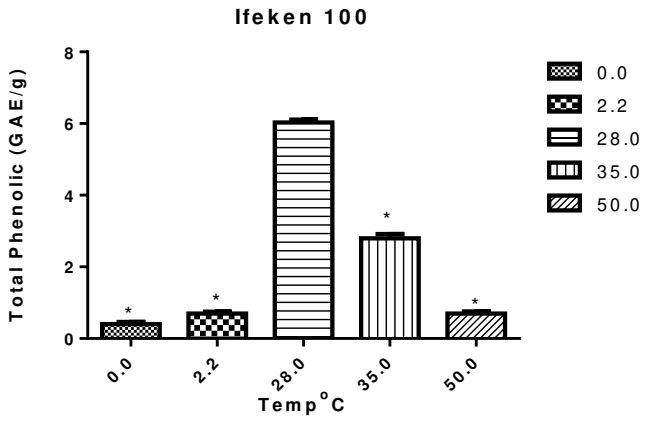

Figure 1.Effect of temperature on Total phenolics of Kenaf (Ifeken 100).

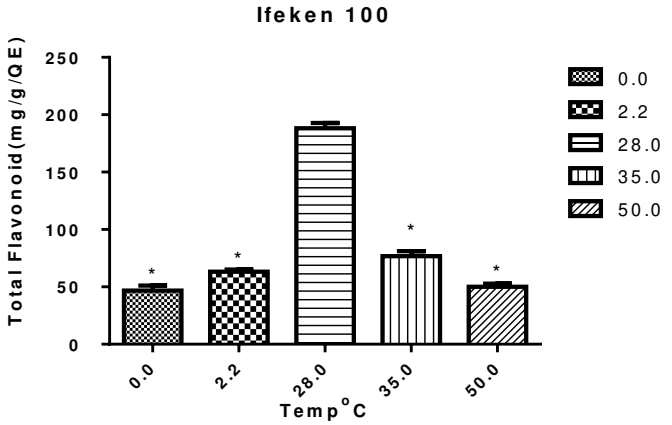

Figure 2.Effect of temperature on Total flavonoid of Kenaf (Ifeken 100).

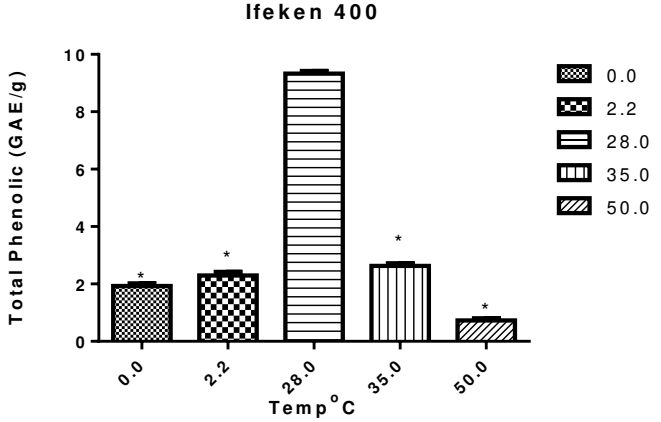

Figure 3.Effect of temperature on Total phenolics of Kenaf (Ifeken 400).

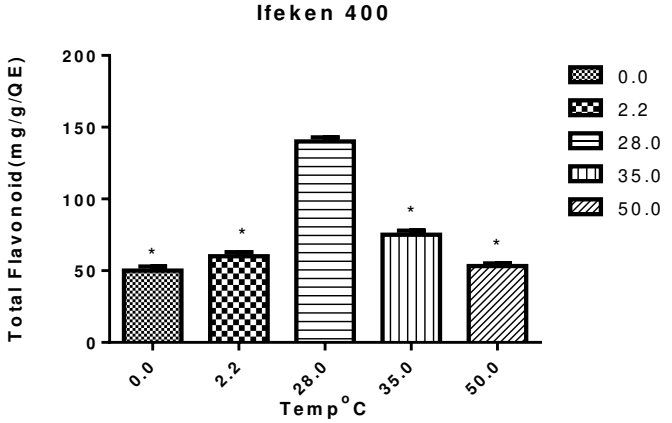

Figure 4.Effect of temperature on Total flavonoid of Kenaf (Ifeken 400).

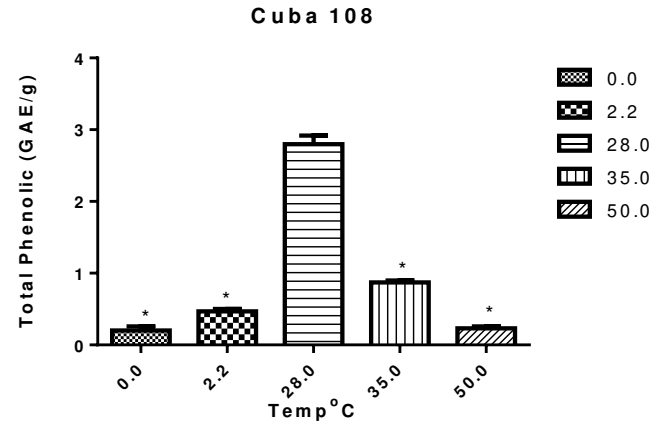

Figure 5.Effect of temperature on Total phenolics of Kenaf (Cuba 108).

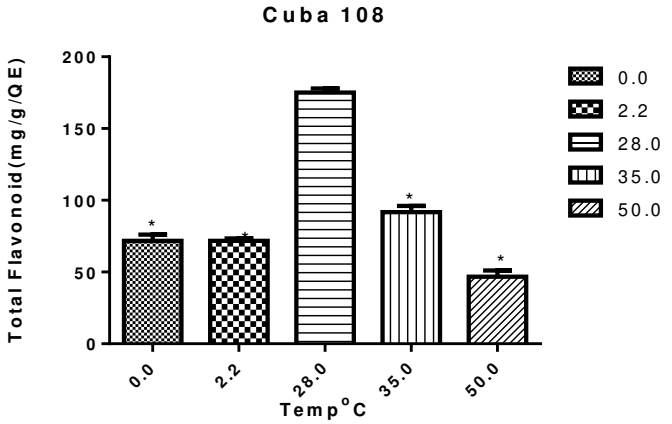

Figure 6.Effect of temperature on Total flavonoid of Kenaf (Cuba 108).

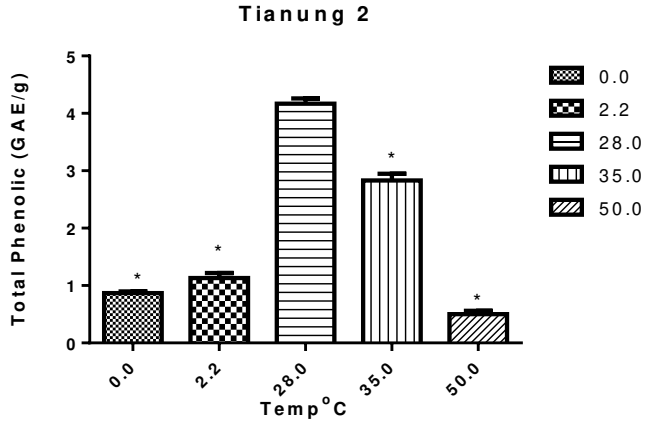

Figure 7.Effect of temperature on Total phenolics of Kenaf (Tianung 2).

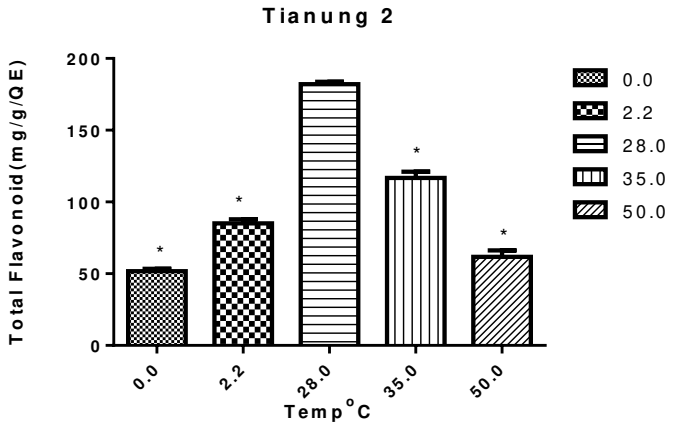

Figure 8.Effect of temperature on Total flavonoid of Kenaf (Tianung 2).

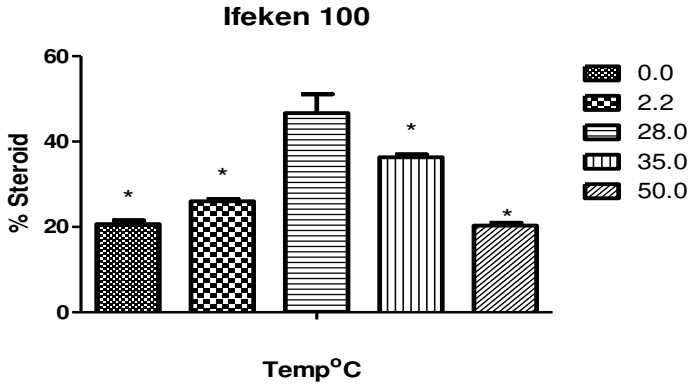

Figure 9. Effect of temperature on steroid of Kenaf(Ifeken100).

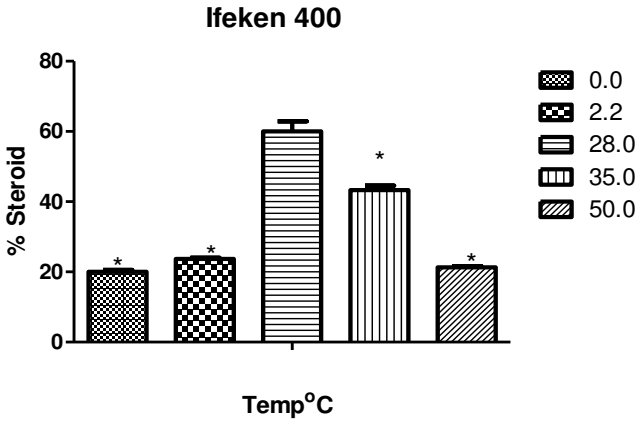

Figure 10. Effect of temperature on steroid of Kenaf(Ifeken400).

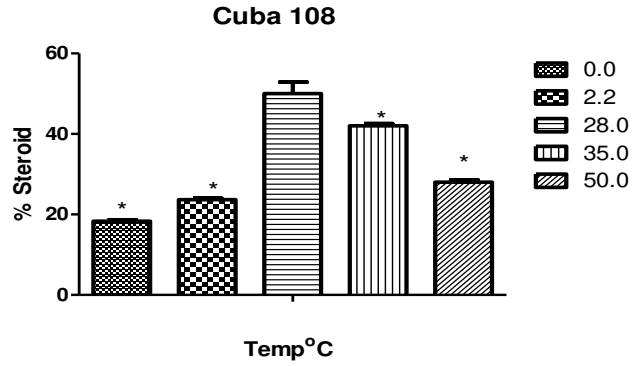

Figure 11. Effect of temperature on steroid of Kenaf(Cuba108).

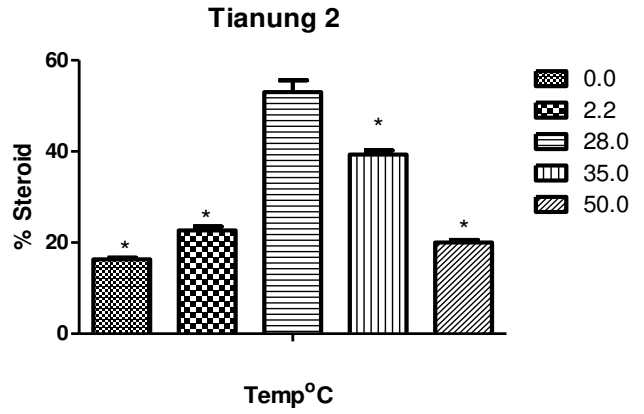

Figure 12. Effect of temperature on steroid of Kenaf(Tianung 2).

Table 5. Effect of temperatures on growth parameter of kenaf(Ifeken 100).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature^{0}C	Seed germination (%)	Seed weight (g)
KS (0)	0.00 ± 0.00*	0.20 ± 0.00*
KS (2.2)	1.67 ± 0.33*	0.34 ± 0.00*
KS (28.0)	65.00 ± 1.73	0.58 ± 0.00
KS (35.0)	48.33 ± 1.67*	0.41 ± 0.00*
KS (50.0)	0.20 ± 0.00*	0.37 ± 0.00*

Table 6.Effect of temperature on growth parameters of kenaf (Ifeken 400).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature^{0}C	Seed germination (%)	Seed weight (g)
KS (0)	0.00 ± 0.00*	0.21 ± 0.00*
KS (2.2)	1.00 ± 0.58*	0.36 ± 0.00*
KS (28.0)	80.00 ± 0.58	0.59 ± 0.00
KS (35.0)	50.00 ± 1.15*	0.47 ± 0.00*
KS (50.0)	0.6 ± 0.33*	0.30 ± 0.00*

Table 7. Effect of temperature on growth parameters of kenaf (Cuba 108).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature ^{0}C	Seed germination (%)	Seed weight (g)
KS (0)	0.00 ± 0.00*	0.24 ± 0.00*
KS (2.2)	0.67 ± 0.33*	0.38 ± 0.00*
KS (28.0)	50.00 ± 0.58	0.47 ± 0.00
KS (35.0)	40.00 ± 0.58*	0.52 ± 0.00*
KS (50.0)	0.33 ± 0.33c	0.41 ± 0.00c

Table 8.Effect of temperatures on growth parameters of kenaf (Tianung 2).Values are Mean ± Standard error, n= 3, Means with *significantly different from control (28°C) ns=nonsignificant.

Temperature ^{0}C	Seed germination (%)	Seed weight (g)
KS (0)	0.00 ± 0.00*	0.28 ± 0.00*
KS (2.2)	0.67 ± 0.33*	0.39 ± 0.00*
KS (28.0)	56.67 ± 0.76	0.52 ± 0.00
KS (35.0)	40.00 ± 1.15*	0.49 ± 0.00*
KS (50.0)	0.67 ± 0.33*	0.36 ± 0.00*

As conclusion,the present study shows that low seed viability commonly associated with kenaf seed under storage condition was not only due to oil content alone but in combination with deterioration in physical and chemical compositions like seed weight, crude protein, crude fibre, fat, moisture , carbohydrate and ash which majorly contributed to reduction in seed viability. Therefore it is necessary to determine both physical and chemical properties of kenaf seed under storage because of its relevance to its viability which is a function of growth and development in kenaf production

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