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Oil Content and Fatty Acid Composition of Five Linseed Varieties Grown In Two Agro-Ecological Locations of Kenya

Ms. Lilian W. Kariuki[1], Dr. Arnold N. Onyango[2], Prof. Peter W. Masinde[3], Prof. Stephen M.Githiri[4], Dr. Kenneth Ogila[5]

Abstract

Linseed oil is rich in the health promoting fatty acids, alpha-linolenic acid (omega-3) and oleic acid (omega-9), and is increasingly being used as a nutraceutical. However, there is limited linseed production in Kenya today, necessitating an increase in its production while ensuring a high content of the two fatty acids. This study aimed to determine the oil content and fatty acid profiles of five linseed varieties grown in a relatively warmer location, Juja, with that grown in a cooler location, Njoro, in two seasons (February-June and July-December); as well as the effect of nitrogen fertilizer application on linseed oil contents and fatty acid profiles. Mean oil content was slightly but significantly higher ($p < 0.05$) in Juja than in Njoro (34.8% versus 32.5 %, respectively). Mean oleic acid content was significantly higher in Juja (24.2 %) than Njoro (19.0 %), while alpha-linolenic acid was significantly higher in Njoro (48.5%) than Juja (44.2%). There were inter-varietal differences in both oil content and fatty acid profiles, and the significance of these differences were both season and location-dependent. Nitrogen fertilizer application had minimal effect on oil contents and fatty acid profiles. In conclusion, good quality linseed can be produced in both agro-ecological locations.

Keywords: Linseed, oil content, alpha linolenic acid, oleic acid, nitrogen fertilizer

1. Introduction

The linseed plant (*Linumusitatissimum*L.) is widely cultivated for fiber and oil (Bayrak*et al*., 2010). Linseed has a high oil content of about20-40%, and α-linolenic acid, anω-3 fatty acid, makes up 30-60% of its total fatty acid content (Green and Marshall, 1981; Bean and Leeson, 2001; Pali and Mehta, 2014). Other major unsaturated fatty acids in linseed oil are oleic acid (ω-9) which accounts for up-to 30% and linoleic acid (ω-6) which accounts for up-to 20% of the fatty acids (Green and Marshall, 1981; Bean and Leeson, 2001; Pali and Mehta, 2014). While ω-6 fatty acids such as linoleic acid and arachidonic acid are converted in human tissues to pro-inflammatory metabolites that promote the development of physiological disorders such as atherosclerosis, cancer and diabetes, the ω-3 fatty acids are converted to anti-inflammatory products with various health benefits (Deckelbaum, R. J., Torrejon, C., 2012). Oleic acid also has such health benefits as increasing the levels of antiaging metabolites (Enot et al, 2015), a hypotensive effect, activity against breast, bladder, prostate and oesophageal cancers (Moon et al, 2014), and the ability to promote adaptability of cells to oxidative stress (Haeiwa et al, 2014).

[1]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology. P.O Box 62000-00200 Nairobi-Kenya
[2]Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology. P.O Box 62000-00200 Nairobi-Kenya
[3]Meru University of Science and Technology. P.O. Box 972-60200 Meru-Kenya.
[4]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology. P.O Box 62000-00200 Nairobi-Kenya
[5]Department of Zoology, Jomo Kenyatta University of Agriculture and Technology. P.O Box 62000-00200 Nairobi-Kenya

Modern diets tend to have a high ratio of ω-6 toω-3 fatty acids, and this has been suggested to contribute to the present rise in non-communicable diseases (Patterson et al, 2012), which were previously considered to affect only high income countries, but are now a heavy burden on low income countries as well (Kankeu et al, 2013). Hence there is a need to increase the amounts of health-promoting ω-3 fatty acids and oleic acid (ω-9) in the diet, and this may be partly achieved by increasing the production and consumption of linseed oil.

About 2.3 million tonnes of linseed was produced worldwide in 2013, of which Canada, the leading producer contributed 712,000 tonnes (FAOSTAT, 2013). In the same year, the leading linseed producer in Africa, Ethiopia, produced 104,948 tonnes, while the second producer, Kenya, produced a meagre 1000 tonnes (FAOSTAT, 2013). In Kenya, linseed production is limited to high altitude areas with cool climate, where there is pressure for land to grow other high value crops such as tea, coffee and wheat. Thus there is a need to explore linseed production in other agro-ecological zones. This requires, among others, selection of varieties that are suited for different egro-ecological zones, and determination of the effects of various agronomic conditions and practices on linseed oil quality. For linseed, two key quality parameters are oil content and fatty acid profiles, and there is literature indicating that the latter is particularly affected by temperature (Lanna et al, 2005).The purpose of this study was to (i) determine and compare the oil contents and fatty acid profiles of linseeds of five different varieties grown in two different agro-ecological zones, (ii) determine the effect of season on the oil content and fatty acid profiles of the linseed varieties and (iii) determine the effect of nitrogen fertilizer application on the oil contents and fatty acid profiles of the linseed varieties.

2.0 Materials and Methods

2.1 Cultivation of linseeds

Five linseed varieties namely Summit, Raja, S19/21, Jahwar, S19/12 were obtained from the Kenya Agricultural Research Station, Njoro. They were grown in the farm of Jomo Kenyatta University of Agriculture and Technology, which is located in Juja (altitude of 1416 m above sea level, (1° 10' S, 37° 7' E) and Njoro (altitude of 2423m above sea level (-0^{o} 29'S, 35^{o} 56'E latitude). The experiments were laid out as split plots in randomized complete block design with three N-fertilizer levels{0Kg N/ha (control), 75Kg N/ha and 150Kg N/ha} as main plots, and five linseed varieties {Summit (V1), S19/21 (V2),Raja (V3), Jawhar (V4) and S19/12 (V5)} as sub-plots in two seasons (February-June 2012 and July-December 2012). Land was ploughed to a fine tilth and plots measuring 3mx 3m prepared. Drills of 20cm apart and 2cm deep were made along each of the plots. Seeds were then evenly spread along the drills and covered with a thin layer of soil. Germinated seeds were kept weed free. Application of treatment N-fertilizer was done as CAN (26% N) and commenced thirty days after planting and was done in three equal splits, being 2 weeks apart from each other. After maturity, the plants were harvested and dried out in the sun before extracting seeds by threshing. Seeds from each plot were bulked and weighed to determine total plot yield. A sample of 5g seed weight was drawn from each bulked seed per plot. This was ground into fine powder then used for determination of oil content.

2.2 Determination of oil content and fatty acid profiles

Oil content was determined by the AOAC method (1996). Fatty acids profiles were determined by gas chromatography. Briefly, fatty acids were converted to their methyl esters by the acid-catalysedmethanolysis process. Ten miligrams of the extracted oil was refluxed with 4ml methanolicHCl (consisting of 1.5 ml of 8% HCl in 7.5 ml of methanol) for one and a half hours. After cooling, the methyl esters were extracted with hexane, and the hexane layer dried over anhydrous sodium sulfate. A fifth of a microlitre of the extract was injected into a 9A Shimadzu gas chromatograph fitted with a 3m, 15% DEGS column and a FID detector. The carrier gas was nitrogen flowing at the speed of 20 m/s. The injection port and column were maintained at 170°C while the detector temperature was 240^{o}C. Qualitative interpretationof chromatogram was done through comparison of retention times of sample fatty acid methyl esters (FAMES) with those of FAME standards. Calculation of the percentage of fatty acids was based on peak areas.

2.3 Data analysis

Data on percent oil content and proportions of fatty acids was subjected to analysis of variance (ANOVA) using PROC GLM in SAS 9.1.3 portable version and the means were separated using LSD procedure at the 0.05 level of significance. Levels of significance between the means of seasons as well as sites were determined using paired t-test procedure in SAS. Graphs were plotted using SigmaPlot 12.0 while tables were inserted in word.

3.0 Resultsand Discussion

3.1 Monthly temperatures in Juja and Njoro

Table 1 shows the average monthly temperatures in the two locations during the study period. Njoro had lower temperatures than Juja during the entire study period. In both locations, the average temperatures were higher in the February-June season than in the July–December season. However, the monthly temperatures were higher at the beginning of the February-June season and decreased towards the end of the season. On the other hand, the July–December temperatures were lower at the beginning of the season and increased towards the end. Hence the temperatures during the seed filling stage were comparable in both seasons (late April-early June and late October – earlyDecember).

Table 1: Mean monthly temperatures in Juja and Njoro during the study period

Month	Njoro	Juja
February	17.9	19.7
March	18.3	20.2
April	18.1	20.1
May	17.1	18.9
June	15.9	17.3
July	15.4	16.5
August	15.5	16.7
September	16.3	18.3
October	17.1	19.5
November	17.0	19.2
December	17.1	19.0

3.2 Oil content of five linseed varieties in two seasons, two locations

During the February-June 2012 season in Juja, oil contents of the five linseed varieties ranged from 33.7% in Summit to 38. 5% in Raja (Fig 1a). However, the inter-varietal differences in oil content were not significant ($p > 0.05$). In the July-December 2012 season, the oil contents reduced, ranging from 32.7 % in Raja to 36.3 % in S19/21 (Fig 1b). In the July-December season, S19/21 produced significantly higher ($p<0.05$)oil content than the other varieties (Fig 1b).The fact that Raja had the highest oil content in the February-July season and the lowest in the July-December season indicates a greater sensitivity of this variety to environmental conditions. Similarly to Juja, the oil contents in Njoro were higher during the February-June season (31.3 -38.4%) than the July-December season (28.0 % -31.3%) (Fig 1c and 1d). However, in this location, inter-varietal differences in oil contents were significant in both seasons ($p<0.05$). S19/21 had the highest oil contents in both seasons, while the least oil content was produced by Jahwar (31.3 %) and Raja (28.6 %) in the two seasons, respectively. The average oil content of the five varieties in the two seasons was slightly higher in Juja than Njoro, and this difference was significant (Table 2). However, inter-season differences in oil content were not significant in both Juja and Njoro (Table 3).

Some studies on the oil contents of linseed varieties grown under different agro-ecological environments have reported mean values ranging from 23- 45.7 % (Diederichsen and Fu 2008, Bayrak*et al.,* 2010; El-Beltagi*et al.,* 2007; El-Beltagi*et al.,* 2011; Green and Marshall, 1981). The oil contents of the five varieties in the present study fell within this range (Figures 1 and 2). Canvin (1965) found that linseed grown under lower temperatures gave higher oil contents than that grown in higher temperatures. Because Njoro had lower average temperatures than Juja, oil contents would be expected to be higher at the former location than the latter. However, this was not the case (Table 2), showing that some other factor besides temperature also contributes to the oil content. In both locations, the temperatures during seed filling were comparable in the two seasons, yet oil contents were higher in the February-June season than the July-December season, even if the inter-season differences were insignificant.

The reason for this is not clear, but it might be linked to the fact that in the February-June season, the daily temperatures during seed filling period (late April-early June) were on a declining trend while those in the July-December season (late October-early December) were more or less constant.

3.3 Effects of nitrogen fertilizer on oil contents

In the February-June season in Juja, application of N fertilizer at 75 Kg/ Ha led to a significantly higher oil content ($p < 0.05$) than the control (0 Kg/ Ha) or application of the fertilizer at 150 Kg/ Ha (Figure 2a). However, there was no significant difference in oil content between the control and the 150Kg/ Ha treatment. In the July – December season, the control gave significantly higher oil content ($p< 0.05$) than the 75Kg/Ha or 150 Kg/ Ha treatments (Fig 2b). In Njoro, the control (0 N/ Kg) gave significantly higher oil content ($p<0.05$) during the February-June season than the 75KgN/ha treatment (Figure 2c). During the July-December 2012 season however, there were no significant differences in the oil content of seeds cultivated under different levels of nitrogen application (Fig 2d). Nevertheless, the highest N level gave higher oil content (Fig 2d).

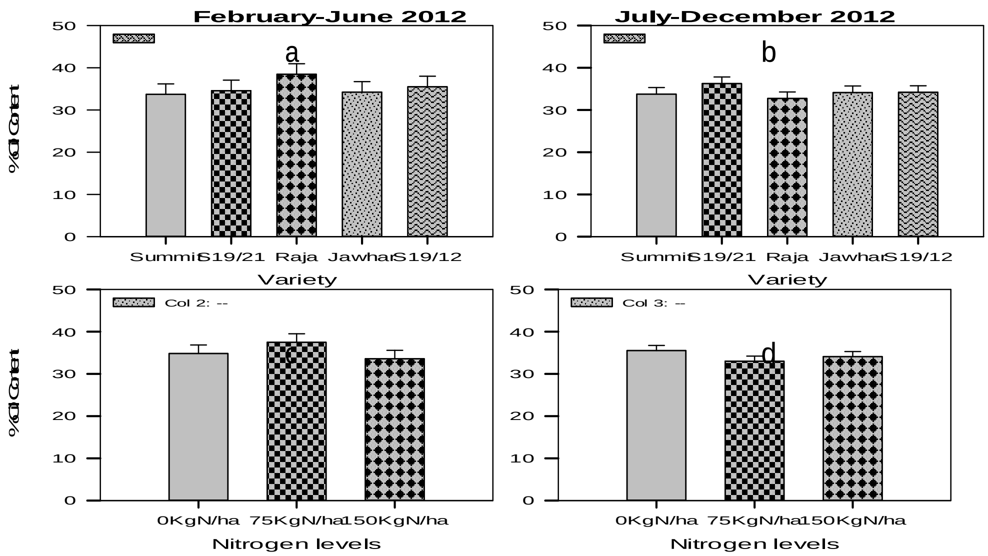

Figure 1: Oil content from five linseed varieties grown in Juja (a,b) under three nitrogen levels (c,d) in two different seasons

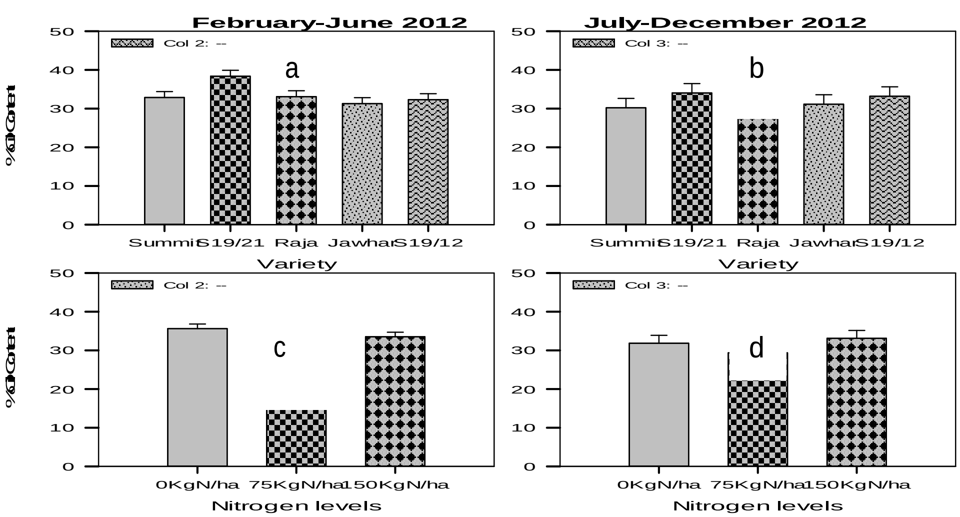

Figure 2: Oil content from five linseed varieties grown in Njoro (a,b) under three nitrogen levels (c,d) in two different seasons

Table 2: Comparison of the mean oil contents (%), and proportions (%) of oleic acid and alpha-linolenicacid (ALA) in linseed produced in Juja and Njoro

Site	Parameter	Mean	Stddev	Std error	T statistic	Prob>t
Juja	Oil content	34.752	1.6272	0.5146	2.824	0.0199
Njoro		32.507	2.6172	0.8276		
Juja	Oleic acid	24.219	1.4186	0.4486	8.391	<.0001
Njoro		19.003	2.1082	0.6667		
Juja	ALA	44.235	2.9901	0.9455	-3.904	0.0036
Njoro		48.486	1.8527	0.5859		

Table 3: Comparison ofmean oil contents (%) and proportions (%) of oleic acid and alpha-linolenic acid (ALA) in linseed grown in the February-June 2012 season (season 1) and July-December 2012 season (season 2) in Juja and Njoro

Site	Parameter	Season	Mean	Stddev	Std error	T statistic	Prob>t
Juja	Oil content	1	35.292	1.8895	0.845	0.858	0.4391
		2	34.212	1.2876	0.5758		
	Oleic acid	1	23.884	1.2392	0.5542	-1.299	0.2637
		2	24.554	1.6468	0.7365		
	ALA (%)	1	45.174	1.705	0.7625	0.790	0.4737
		2	43.376	3.9197	1.753		
Njoro	Oil content	1	33.584	2.7576	1.2333	1.973	0.1197
		2	31.43	2.2154	0.9907		
	Oleic acid	1	17.684	2.2082	0.9875	-2.812	0.0482
		2	20.322	0.8805	0.3938		
	ALA	1	49.816	1.2601	0.5635	3.120	0.0355
		2	47.156	1.3087	0.5853		

Berti et al (2008) reported that N fertilization up to 200Kg N/Ha in South Central Chile increased oil content of linseed. Similarly, Ibrahim (2009) reported that increasing N fertilization of linseed from 107 -179 Kg/Ha led to increased oil content. On the other hand, Rahimi et al (2011) found that up to 50 Kg/Ha of N fertilizer had no effect on linseed oil content, while 100Kg N/ha and above reduced oil content. In the present study, the effect of nitrogen treatment was not only dependent on the rate of application but also on the location and the season. For example, during the February-June season, N application at 75KgN /Ha increased oil content in Juja, but reduced oil content in Njoro. In both locations and seasons, there was no benefit of N application at 150Kg/Ha.

3.4 Fatty acid profiles of five linseed varieties in two locations, two seasons

The major fatty acids in the seeds of the five varieties were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and α-linolenic (18:3) (Tables 4and 5). Wide variations in the relative proportions of the five main fatty acids in linseed have been reported (Green and Marshall, 1981; Bean and Leeson, 2001; Pali and Mehta, 2014), with such values as 1.3-7.6 % stearic acid, 5.0-9.9 % palmitic acid, 10.4-20.9 % linoleic, 13.3-35% oleic and 33.1 to 63.1% alpha-linolenic acids. Similar levels of these fatty acids were found in the present study (Tables 4and 5).

Table 4: Fatty acid (%) profiles of five linseed varieties grown in Juja during February-June and July-December 2012 (Season 1 and Season 2 respectively)

		Fatty acid % and ω3:ω6 ratio							
Season1	**Variety**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**Others**	**ω3:ω6**	**ω3+ω9**
	Summit	8.71a	2.31a	23.32a	18.18a	45.23a	2.25	2.5:1	68.55
	S19/21	9.03a	1.85a	24.05a	19.32a	42.61a	3.14	2.2:1	66.66
	Raja	7.81a	1.27a	22.06a	18.10a	46.91a	3.85	2.6:1	68.97
	Jawhar	7.85a	1.49a	25.02a	17.13a	46.36a	2.15	2.7:1	71.38
	S19/12	8.40a	1.84a	24.97a	17.66a	44.76a	2.37	2.5:1	69.73
	Mean	8.36	1.75	23.88	18.01	45.17	2.75	2.5:1	69.06
Season2	**Summit**	8.15a	1.70a	24.01a	18.27a	45.71a	2.16	2.5:1	69.72
	S19/21	7.13a	1.98a	23.17a	17.31a	48.22a	2.19	2.8:1	71.39
	Raja	8.81a	2.42a	23.34a	19.08a	43.98a	2.37	2.3:1	67.32
	Jawhar	8.81a	2.58a	27.18b	19.30a	40.46a	1.67	2.1:1	67.64
	S19/12	8.88a	2.62a	25.07b	17.62a	38.51b	7.30	2.2:1	63.58
	Mean	8.36	2.26	24.55	18.32	43.37	3.14	2.4:1	67.93

Values with same alphabet in each season are not significantly different along the column while values followed by different alphabets are significantly different at 5%probability level.

Table 5: Fatty acid (%) profiles of five linseed varieties grown in Njoro during February-June and July-December 2012 (Season 1 and Season 2 respectively)

Values with same alphabet in each season are not significantly different along the column while values followed by different alphabets are significantly different at 5%probability level.

		Fatty acid % and ω3:ω6 ratio							
Season 1	**Variety**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**Others**	**ω3:ω6**	**ω3+ω9**
	Summit	9.55a	2.7a	13.88b	16.22b	51.3a	6.35	3.2:1	65.18
	S19/21	8.18a	2.07a	18ab	17.84ab	51.05a	2.86	2.9:1	69.05
	Raja	9.35a	2.95a	18.51ab	17.92ab	49.0a	2.27	2.7:1	67.51
	Jawhar	8.57a	2.21a	19.61a	18.78a	48.59a	2.24	2.6:1	68.2
	S19/12	8.9a	2.28a	18.42ab	18.33ab	49.14a	2.93	2.7:1	67.56
	Mean	8.91	2.44	17.68	17.82	49.82	3.33	2.8:1	67.5
Season 2	**Summit**	9.49a	3.76a	20.17a	19.39a	45.75a	1.44	2.4:1	65.92
	S19/21	8.72a	3.51a	19.11a	18.75a	48.07a	1.84	2.7:1	67.18
	Raja	7.94a	3.45a	20.96a	18.16a	47.11a	2.38	2.6:1	68.07
	Jawhar	9.09a	3.08a	21.37a	18.62a	46.03a	1.81	2.5:1	67.4
	S19/12	8.27a	3.17a	20.00a	17.57a	48.82a	2.17	2.8:1	68.82
	Mean	8.70	3.39	20.3	18.50	47.16	1.93	2.6:1	67.49

During the February-June season in Juja, the linseed varieties did not significantly differ in their fatty acid composition (Table 4). However, in the July-December season, Jahwar and S19/12 had significantly higher oleic acid (18:1) content than the other varieties (Table 4).

In Njoro, there were significant inter-varietal differences in oleic acid (18:1) and linoleic acid (18:2) contents during the February–June season (Table 5). However, there were no significant differences in their fatty acid composition in the July-December Season (Table 5).

The major linseed fatty acids of interest for improving human health are α-linolenc acid and oleic acid. Significantly higher α-linolenic acid contents were obtained in Njoro than Juja, while the opposite was true for oleic acid (Table 2). This may be attributed to the lower temperatures in Njoro than Juja in accordance with previous findings of Canvin (1965) and Gallardo et al (2014) that higher temperatures result in higher oleic acid and lower linolenic acid contents of linseed. Similar results were obtained for rapeseed by Deng and Scarth (1998). The production of higher amounts of α-linolenic acid under lower temperatures may be explained by the need for greater unsaturation of membrane phospholipids, which is important for maintaining membrane fluidity (Lana et al, 2005). Oleic acid is a precursor of α-linolenic acid through the successive actions of oleatedesaturase and linoleatedesaturase (Rabiei et al, 2007), and this may explain the negative relationship between oleic acid and α-linolenic acid. In both locations, there was a slightly higher average oleic acid content in the July-December season than the February-June season, while the opposite was true for α-linolenic acid, with the inter-season differences in these fatty acids being significant in Njoro but not in Juja (Table 3).

The February-June season that gave higher oil content (vide supra) also gave higher α-linolenic acid content. This is related to the finding of Pali and Mehta (2014) that oil content in linseed was positively related withα-linolenic acid content. The same phenomenon manifests in the result that in Juja, Raja variety had the highest oil content and highest linolenic acid content in the February-June season, while S19/21 had the highest oil content and highest linolenic acid content in the July-December season (Table 4).

In both locations, the mean ω3:ω6 ratio was higher in the February-June season than the July-December season (Tables 4 and 5), which is due to the higher amounts of α-linolenic acid contents in the former season than the latter. While the ω3:ω6 ratio was higher in Njoro than in Juja in both seasons, the combined percentage of α-linolenic acid and oleic acid (ω3 + ω9) was higher in Juja than Njoro. Therefore linseed produced in both locations can be considered to be of good value for use as a functional food.

3.5 Effect of nitrogen fertilizer application on fatty acid composition of linseed varieties

As shown in Table 6, nitrogen application did not significantly affect the fatty acid profiles of the linseed oil in Juja in both seasons. Similar results were obtained in Njoro (data not shown).

Table 6: Effect of fertilizer application on fatty acid profiles of linseed in Juja

		Fatty acid (%) and □3:□6 ratio							
	KgN/Ha	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**Others**	**ω3:ω6**	**ω3 + ω9**
Season1	**0**	8.71a	1.73a	23.04a	18.6a	45.48a	2.45	2.4:1	68.52
	75	9.03a	1.37a	24.40a	17.91a	44.41a	2.88	2.5:1	65.81
	150	7.81a					2.45		69.86
			2.16a	24.22a	17.72a	45.64a		2.6:1	
Season 2	**0**	7.85a	2.37a	24.43a	17.4a	43.98a	3.97	2.5:1	68.41
	75	8.40a	2.45a	26.11a	18.21a	42.83a	2.00	2.4:1	68.94
	150	8.71a	2.22a	25.13a	19.34a	43.32a	1.28	2.2:1	68.45

Values with same alphabet in each season are not significantly different along the column while values followed by different alphabets are significantly different at 5%probability level

4.0. Conclusions

From the results obtained in this study, it can be concluded that linseed grown in Juja and Njoro has acceptable oil content and fatty acid composition. Similar work in other locations will help to determine the potential for linseed production as a functional food in other agro-ecological regions. The oil contents of the different varieties were influenced to different extents by the seasons. Thus, selection of a variety for cultivation should be made with due consideration of the season. Some inter-varietal differences in fatty acid profiles were observed, but whether these differences were significant depended both on the fatty acid, the location and the season. Nitrogen fertilizer application was found not to lead to much improvement, if any, in the oil content and quality of linseed in both locations tested. Further work should be done to establish the effect of other nutrients on linseed production.

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