Journal of Medicinal and Industrial Plant Siences (2025) 3 (1): 66- 79
ISSN: 2959 – 121X
DOI: https://doi.org/10.32894/MEDIP.25.3.6

Seeds and oil properties of some Niger (*Guizotia abyssinica* cass) cultivars.

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ABSTRACT

Seed specimen was collected from field trial to the evaluation of three Niger cultivars based on their seed physical properties, oil chemical composition, and anatomical characteristics. The results showed that significant genotypic variability was observed across these varieties and their studied physical, chemical, and anatomical traits. The cultivars parameters referred that the mature seeds are usually composed of three cell layers (seed coat, seed embryo, and Endosperm). The results of lab analysis revealed substantial variability, with crude protein content ranging from (21.32 to 23.45) g/100 g^{-1}, ash content from (3.03 to 4.45) g/100 g^{-1}, while the highest value for fiber content was (21.40) g/100 g^{-1} that obtained in the Karal cultivar. Microscopic images indicated that seeds from the Animax cultivar had maximum values for three axial and area compared to the other cultivars. The geometric mean diameter and arithmetic diameter ranged from (0.70 to 1.76) and (0.23 to 0.59) mm respectively. Significant differences were detected in fatty acid profiles among the evaluated cultivars. 'Benglanuglue' cultivar exhibited the highest concentrations of oleic and linolenic acids of (8.12 and 18.43) %, while the 'Animax' cultivar demonstrated the highest levels of linoleic acid (24.16%. These findings suggest that both cultivars possess favorable fatty acid compositions for potential culinary applications. Among the evaluated cultivars, 'Karal' exhibited the most favorable profile for direct culinary applications, characterized by the lowest palmitic acid content. Furthermore, both 'Karal' and 'Animax' cultivars demonstrated reduced levels of palmitic and stearic acids. All evaluated cultivars exhibit potential for contributing on the enhancement of oil quality across the Kurdistan region of Iraq. Consequently, these cultivars are required to test over other sites belong to the given region.

KEY WORDS: Oil; Protein; Dimensional properties; Germination; Fatty acids composition.

Received: 30/01/2025 ; **Accepted**: 19/02/2024 ; **Available online:** 31/03/2025

صفات البذور والزيت لبعض اصناف النايجر(*Guizotia abyssinica* Cass)

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الملخص

تضمنت الدراسة تقييم ثلاثة أصناف من النيجر بناءً على الخواص الفيزيائية و للبذور والتركيب الكيميائي للزيت. أشارت النتائج إلى ملاحظة تباين جيني كبير عبر هذه الأصناف من خلال دراسة خصائصها الفيزيائية والكيميائية والتشريحية المدروسة. أشارت معايير الأصناف إلى أن البذور الناضجة تتكون عادةً من ثلاث طبقات من الخلايا (غلاف البذرة وجنين البذرة والسويداء). كشفت نتائج التحليل المختبري عن تباين كبير، حيث تراوح محتوى البروتين الخام من (21.32 إلى 23.45) جم / 100 جم، ومحتوى الرماد من (3.03 إلى 4.45) جم / 100 جم، بينما كانت أعلى قيمة لمحتوى الألياف (21.40) جم / 100 جم تم الحصول عليها في صنف كارال. كان لبذور صنف أنيماكس أقصى قيم للمحور الثلاثي والمساحة مقارنة بالأصناف الأخرى. تراوح متوسط القطر الهندسي والقطر الحسابي من (70 إلى 176) و (0.16 إلى0.37) ملم على التوالي .تم الكشف عن فروق ذات دلالة إحصائية في ملفات الأحماض الدهنية ضمن الأصناف المقيمة. أظهر الصنف **'Benglanuglue'** أعلى تركيزات من الأحماض الأوليك واللينولينيك% (8.12, 18.43)، في حين أظهر الصنف **'Animax'** أعلى مستويات حمض اللينوليك% 24.16. تشير هذه النتائج إلى أن كلا الصنفين يمتلكان تركيبات أحماض دهنية مواتية للتطبيقات الطهوية المحتملة. من بين الأصناف المقيمة، أظهر **'Karal'** الملف الأكثر ملاءمة والذي يتميز بأقل محتوى من حمض البالمتيك. علاوة على ذلك، أظهر كل من صنفي **'Karal'** و**'Animax'** مستويات منخفضة من أحماض البالمتيك والستياريك. تُظهر جميع الأصناف المقيمة إمكانية المساهمة في

تحسين جودة الزيت داخل إقليم كردستان.

الكلمات المفتاحية: الزيت، البروتين، خصائص الابعاد، الانبات، تكوين الاحماض الدهنية .

INTRODUCTION

Niger (*Guizotia abyssinica* Cass.), a member of the Asteraceae family, is an economically significant oilseed crop with a long history of cultivation in India and Ethiopia, dating back approximately 5000 years (Deme et al., 2017). Niger seeds possess diverse applications, including their utilization as a valuable feeding source for avian species. (Abdulla and Khalaf, 2014) The Niger seed contains up to 40% edible semidrying oil, 20.9 % carbohydrate and 27.8 % protein (Teklewold et al, 2006 and Geleta. et al, 2007). The compositional profile of Niger seeds, encompassing oil, protein, and crude fiber content, is significantly influenced by seed hull thickness. Cultivars characterized by thicker seed hulls generally exhibit reduced oil and protein content while displaying elevated crude fiber levels. Niger seed oil exhibits a fatty acid profile characteristic of Asteraceae family seed oils, notably safflower and sunflower oil, with linoleic acid constituting the predominant fatty acid. Niger seeds are a rich source of high-quality oils, demonstrating exceptional nutritional value. These oils are characterized by a high degree of unsaturation, exceeding 70%, and possess a diverse array of potential medicinal properties (Deme et al., 2017; Mohseni et al., 2020). Niger seeds constitute a valuable oilseed crop, yielding 35-45% oil content alongside 18-20% crude protein, and possessing a characteristically pleasant aroma (Ramadan, 2012; Mohseni et al., 2020). While botanical studies have extensively examined the external morphology of achenes within the Asteraceae family, and some research has focused on embryo and ovule development, comparatively limited attention has been devoted to the intricate structure and ontogeny of the seed coat. However, recent studies (Batista et al., 2015) have highlighted the remarkable diversity observed within Asteraceae seed coats, including the persistence of an exotestal palisade layer (particularly evident in the Cynareae subtribe), variations in exotestal cell wall thickening, and the differential retention of mesophyll and vascular tissues This study aims to evaluate the phenotypic performance and genetic variability of existing Niger cultivars within the Kurdistan Region of Iraq across a suite of traits, including seed physical properties, oil characteristics, and seed oil physicochemical properties. The study will further investigate the phenotypic and genotypic correlations between these traits to facilitate the efficient selection of superior cultivars with enhanced oil yield for future Niger breeding programs.

MATERIALS AND METHODS

A lab trial was undertaken at the College of Agricultural Engineering Sciences, Salahaddin University – Erbil. Three Niger (*Guizotia abyssinica* Cass.) cultivars (Benglanuglue, Karal and Animax) were used in the study these cultivars are registers from Bakrajo Agriculture Research

Center in Sulaimani, Kurdistan-Iraq .

The study was designed as Completely Randomized Design (CRD) using three replicates, the current laboratory experiment included three-line study:

1. Germination behavior of Niger seeds.

2. Anatomy of Niger seeds:

The seed specimen collected were fixed in formalin-acetic-alcohol; and prepare the samples using paraffin method, safranin used and fast green for stain the sections (Al-Khazrajy and Aziz, 1990, Saeed, 2003 and Najmaddin and Mahmood, 2016). Microscopic images were captured using an Olympus light microscope equipped with a color digital camera.

3. Study the physical properties, which included:

a. Seed index: All mass measurements were performed using a high-precision electronic balance (MODEL: HR-200, Serial no.12317438, Japan) capable of achieving a resolution of 0.001 grams.

b. Dimensional properties included [seed length (L), width (W) and thickness (T)]

To ascertain the dimensional and gravimetric properties of the seeds, a random sample of 20 seeds was selected from each replicate. Dimensional measurements, including length, width, and thickness, were meticulously acquired using a Vernier Caliper with a precision of ± 0.01 mm.

Geometric mean diameter (mm) calculated according to the following equation (Mohsenin, 1986):

$$Gmd = (L * W * T)^{\frac{1}{3}} \ldots\ldots\ldots\ldots\ldots\ldots..1$$

Where L, W and T are the length, width and thickness in mm, respectively (Mohsenin,1986)

d. Arithmetic mean diameter (mm) calculated according to the following equation:

$$Da = (L + W + T)/3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. 2$$

e. Sphericity was calculated according to the following equation:

$$\theta = (L * W * T)^{\frac{1}{3}} / L \quad \ldots\ldots\ldots\ldots\ldots\ldots.. 3$$

f. Sphericity %: Seed sphericity was calculated using the equation derived by Mohsenin, (1986), which incorporates of length (L), width (W) and thickness (T) :

$$S = Gmd * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

g. Seed area (mm)2 was calculated by image J software (Schindelin et al., 2015) .

4. Niger seed chemical properties:

a. Extraction of oil:

Niger seeds oil was estimated using oil extraction using the Soxhlet method, following the standardized protocol outlined by the Association of Official Analytical Chemists (A.O.A.C, 1995).

$$Oil\,\% = (Final\ weight\ of\ flask - empty\ weight\ of\ flask) / (weight\ of\ sample)\ X\ 100 \ldots\ldots\ldots 5$$

b. Determination of Protein%

Total nitrogen content was quantified using the standard micro-Kjeldahl digestion method. Subsequently, crude protein content was calculated based on the equation outlined by El-Sahookie and Waheeb .(1990)

$$Protein\,\% = N * 6.25 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ 6$$

c. Determination of soluble Carbohydrate%

The soluble carbohydrate percentage estimated by the following procedure (Allen et al., 1974).

d. Total Ash%

e. Fiber%:

An enzymatic- gravimetric assay kit (sigma Aldrich-TDF- 100) was used for total dietary fiber (TDF) determination. The percentage of total dietary fiber was extracted from the deducted protein and ash weight residue weight (Cunniff and Washington, 1997).

f. Fatty Acids Estimation

Sample preparation adhered to the standardized protocol outlined by the Association of Official Analytical Chemists (A.O.A.C, 1995). This method involves transesterification of the fatty acids through reaction with methanolic potassium hydroxide. A 11.2 g aliquot of potassium hydroxide was dissolved in 100 mL of methanol to prepare the reagent. Subsequently, a 1 g aliquot of lipid was subjected to transesterification by reacting with 8 mL of methanolic potassium hydroxide solution. Following the addition of 5 mL of hexane, the mixture was vigorously agitated for 30 seconds and subsequently allowed to undergo phase separation. The upper hexane phase, enriched with fatty acid methyl esters, was meticulously extracted and subsequently introduced into the Gas Chromatograph (GC) for analytical evaluation .

Chromatographic Analysis of the Samples:

Fatty acid composition was analyzed via gas chromatography-flame ionization detection (GC-FID) employing a Shimadzu GC-2010 instrument. A 30-meter-long capillary column (SE-30, 0.25 mm internal diameter) was utilized for the analysis. Chromatographic conditions were established according to the methodology described by (Zhang et al. 2015).

Show some Chromatographic conditions:

No.	Name	Temperature
1.	Injection area temperature	280 °c
2.	Detector temperature	310 °c
3.	Separator column temperature	120 – 290 (10 °c. min^{-1})
4.	Gas flow rate	100 K pa

The unsaturated fatty acid profile was characterized using High-Performance Liquid Chromatography (HPLC). In addition, samples of oil extracted were taken to identify the variation in fatty acids percentage through the studied factors. The fatty acids analyzed were: Unsaturated Fatty Acids which were included:

Unsaturated fatty acid, which included:

Omega-9 (or) Oleic acid (C18:1)

Omega-6 (or) Linoleic acid (C18:2)

Omega- 3 (or) Linolenic acid (C18:3)

Saturated fatty acids, which included :

Palmitic acid C 16 .

Stearic acid C 18

RESULTS AND DISCUSSION

1. Germination of Niger seeds:

a. Germination % and relative growth index%:

Data represented in the laboratory experiment confirmed that there were highly significant differences between cultivars on most of germination attributes characters, and Animax cultivar super passed the two other cultivars. Dwivedi (2014) stated that the morphology of the seeds is important, this was in harmony with our result that the Animax cultivar recorded highest value in its physical properties (Table 1). Data in Figure (a) shows that cultivars were significantly effect on germination % and relative growth index. The highest values exhibited of Animax cultivar was (74 and 45.89) % while the minimum recorded values of Karal cultivar was (53.11 and 33.57).%

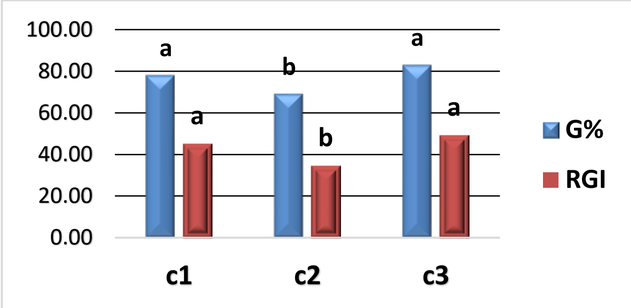

Figure a. Germination% and relative growth index% as affected by Niger cultivars, c1=; c2=; c3=...

b. Germination Index and seedling vigor index :

Figure (b) Shows the highly significant effect between cultivars of germination index and seedling vigor index, maximum value of Animax cultivar (3.34 and 6.67) was recorded for the mentioned traits respectively, while the minimum value (2.27 and 4.42) resulted in Karal cultivar, this may be due to genetic variation between Niger cultivars.

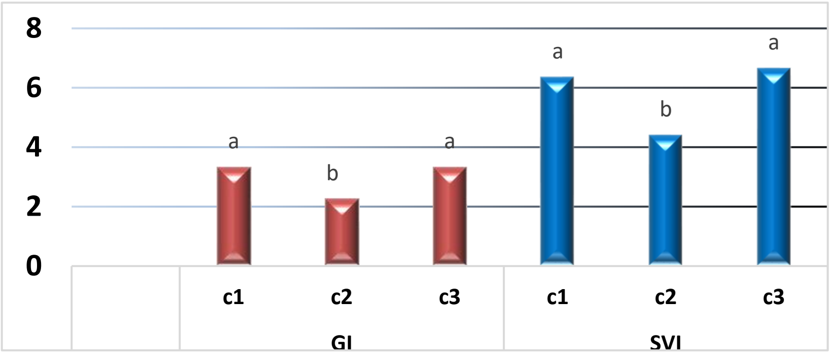

Figure b. Germination index and seedling vigor index as affected by Niger cultivars,c1= ;c2= ;c3=….

c. Peak value of cultivars:

From the results, it was observed in Fig(c), it shows that the peak value was significantly differences between Niger cultivars, the highest values recorded for Animax and Benglanuglue (0.061 and 0.059) while the lowest value for Karal with values of 0.044.

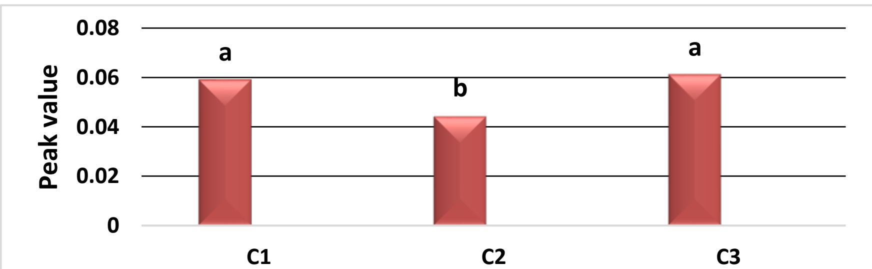

Figure c. Peak values affected by Niger cultivars.

d. Radical and plumule length (cm):

In comparison to the radical and plumule growth of Niger, Data represented in Figure (d) confirmed that there was highly significant effect of cultivars on most of radical and plumule length, Benglanuglue and Animax cultivar super passed Karal cultivars in radical length, highest value recorded was (3.46 and 3.46) cm while minimum values (2.76) cm obtained for Karal cultivar. For shoot length Animax and Karal obtained highest and lowest values which is (5.37 and 4.85) cm. These results in radical and plumule length is different with the results obtained by Dwivedi.(2014)

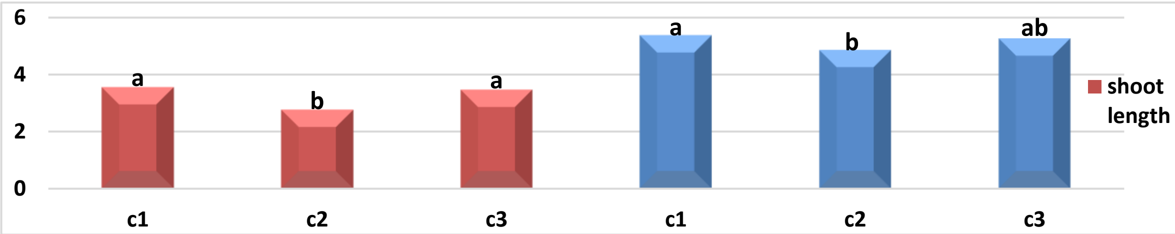

Figure d. Niger cultivars root and shoot length

e. Radical elongation and plumule elongation velocity (cm day^{-1}):

Figure (e) shows that for root elongation velocity traits, there was no significant differences between cultivars, whilst Benglanuglue and Karal cultivars resulted in high and low plumule elongation velocity (cm day^{-1} with values of (0.34 and 0.28) cm day^{-1} respectively .

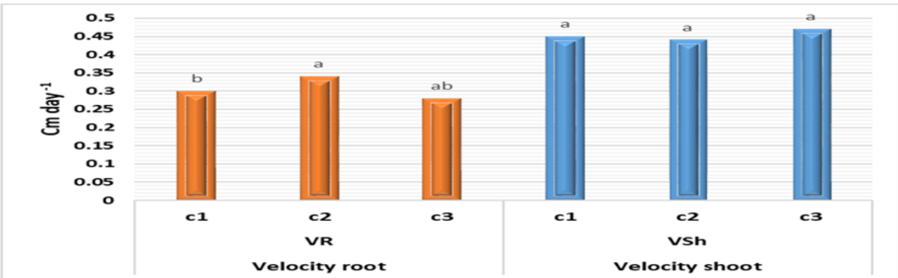

Figure e. Root and shoot velocity elongation (cm/day) affected by Niger cultivars

f. Plumule and radical wet weight (mg):

Data in figure (f) showed that there was significantly affect of plant on radical, plumule and hull plant wet weight, maximum value of radical wet weight was recorded with Animax cultivar (0.25g ,0.80 and 1.04) g respectively, contrasting the minimum value was logged by Karal cultivar (0.16, 0.35 and 0.50) g. These results were mentioned by Hamza et al (2019) that there are differences among cultivars in the mentioned traits according to seed weight (Table 1) which shows that Animax has highest weight comparing with other cultivars).

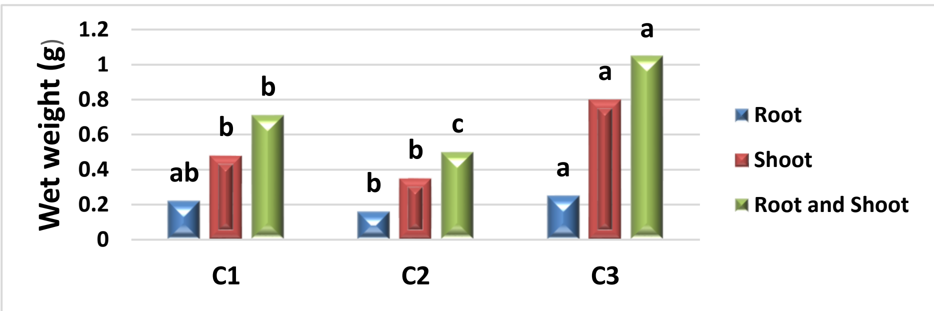

Figure f. Radical, Plumule and all wet weight as affected by Niger cultivars

g. Plumule and radical dry weight (mg):

Different Niger cultivars have significant effect at ($p \leq 0.01$) on root, shoot and holly plant dry weight the highest values were recorded for C3 (Animax cultivar) in values of (0.07,0.21 and 0.28) mg, whilst the lowest dry weight (0.03,0.08 and 0.11) g respectively was obtained for Karal.

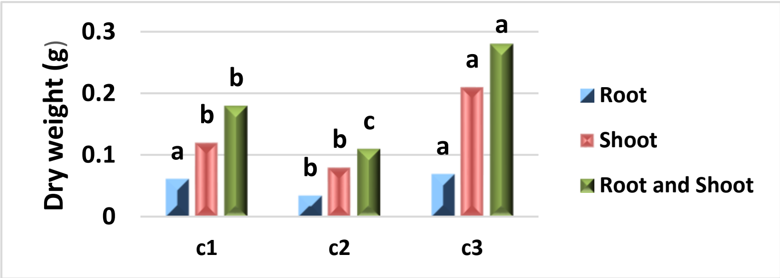

Figure g. Plumule and radical dry weight and all dry weight as affected by Niger cultivars.

2. Anatomy of Niger seeds:

The current study shows the three species of the Niger seeds (Benglanuglue, Karal and Animax) composed of three cell layers (seed coat, seed embryo and Endosperm) with contain the oil droplets; they are different amount between them (Figure h, i and j). The seeds length in Niger Benglanuglue, Karal and Animax respectively (4, 4.5 and 5 mm) (Figure k).

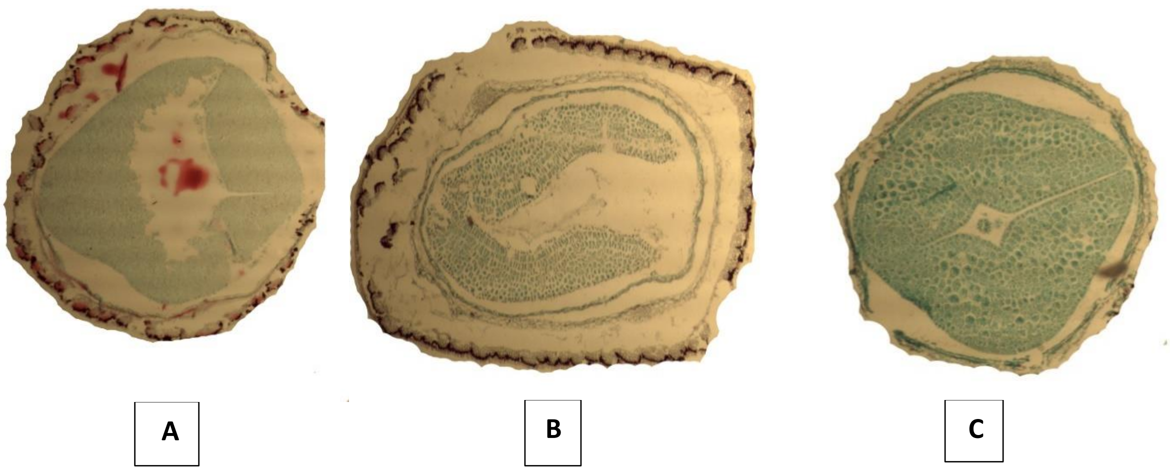

Figure h. Cross sections of seed outlines of the studied taxa of Astaraceae: A- Benglanuglue B- Karal and C- Animax.

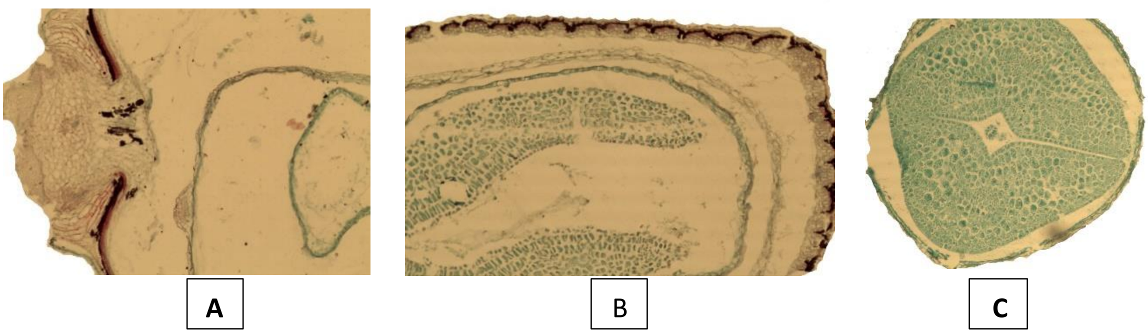

Figure i. Portions of cross section of the studied A- Benglanuglue B-Karal and C- Animax seed.

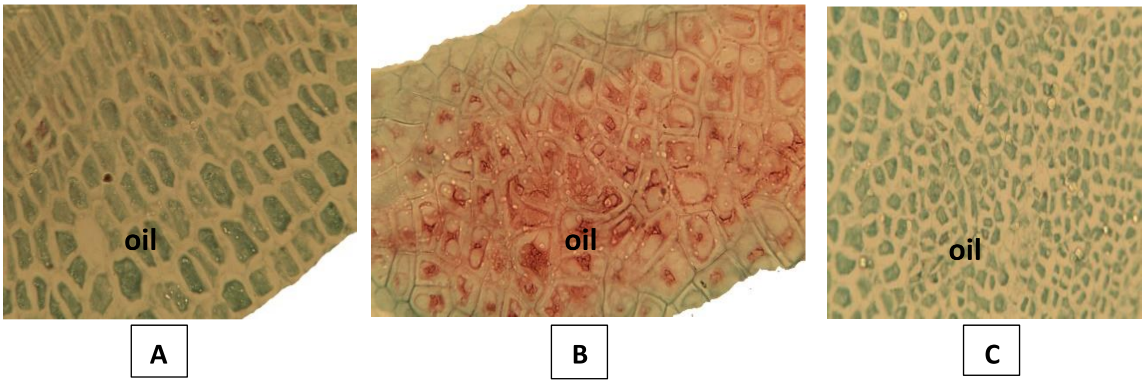

Figure j: Portions of cross section of the studied A- Benglanuglue B- Karal and C- Animax seed.

Figure k. Seed morphology of the studied taxa of Asteraceae A-Benglanuglue B-Karal and C-Animax

3. Physical properties in Niger seeds:

Table 1 shows different seed morphology for (*Guizotia Abyssinica* Cass) cultivars, the results indicate that average length, width of the seed is (4.34 - 4.78) and (0.99 - 0.78) mm for the highest and lowest values of the studied cultivars this is in align with Dwivedi, 2014 who mentioned in his study that average length and width was 5.7 and 1.2 mm. Seeds from Animax cultivar had maximum values for the three axial and area compared to the other cultivars. The phenotypic ratios observed in this study for the investigated cultivars were found to be lower than those reported in previous studies by other researchers (Nazem et al. 2024).

Statistical analysis revealed no significant cultivars differences among the evaluated cultivars for most of the traits assessed, the geometric mean diameter and arithmetic diameter ranged from (1.15 to 1.81) and (2.05 to 3.57) mm respectively. Statistical analysis revealed significant genotypic differences among the evaluated cultivars for most of the traits assessed. However, with respect to seed sphericity which is a specific example of a compactness measure of a shape. the 'Animax' cultivar exhibited the highest value, reaching 0.37 significantly exceeding the values observed in the Benglanuglue cultivar .

The geometric mean diameter and arithmetic diameter ranged from (0.70 to 1.76) and (1.75 to 2.31) mm respectively. Percent of sphericity (Ɵ) ranged from (70 to 176) %.

Table 1 Effect of cultivars on physical properties of Niger seed:

Cultivars	Length (mm)	Width (mm)	Thickness (mm)	Area(mm^2)	Seed index(g)	GMD (mm)	Arithmetic diameter (mm)	Percent of sphericity (%)	Sphericity (Θ)
Benglanuglue	4.36b	0.78 b	0.10 b	0.40b	2.16b	0.70b	1.75b	70b	0.16b
Karal	4.34b	0.82ab	1.20a	0.40b	2.25b	1.62ab	2.12a	162a	0.36a
Animax	4.78a	0.99 a	1.16a	0.70a	3.08a	1.76a	2.31a	176a	0.37a

At 5% of DMRT, means with the same litters for each factor and interaction do not differ significantly

4. Chemical properties in Niger seeds.

As shown in (Table 2) the highest oil content% was 32.47% gained by Benglanuglue cultivar, but the lowest percentage was 24.59 % obtained from Animax cultivar. As indicated by (Liu et al,.2016), the oil percentage varies from medium to high (20 – 45) % depending on the specific variety and prevailing environmental conditions. The experimentally determined oil content values exhibited a high degree of concordance with those reported in prior investigations (Muhammed, 2021). The highest percentages of protein were 24.45% acquired by Animax cultivar, while the lowest percentages were 21.32% obtained from Benglanuglue cultivar. this result agreement with (Gupta et al., 1982 and AL-Hassan et al. 1987) who indicated that the negative correlation between oil content and protein. Ash content exhibited variability, ranging from (3.03 to 4.45) g/100g. The ash content suggests a noteworthy mineral profile, which is lower than that found in Niger meal (7.31%) in a study by (Naz et al. 2021). Ash content in plant samples serves as a proxy for the overall concentration of essential mineral elements within the plant biomass, as previously reported by (Ganogpichayagrai and Suksaard 2020). Notably, the 'Karal' and 'Benglanuglue' cultivars displayed the highest levels of crude fiber (Table 2) .

The current study revealed that fiber content values exceeding previously reported ranges ($9^{-1}3$ g/100 g) by (Rao, 1994). Dietary fiber plays a crucial role in human health, contributing to improved digestive function (e.g., alleviating constipation), mitigating the risk of bowel disorders, lowering cholesterol levels, and promoting overall well-being (Donkor et al., 2022; Sarker and Ercisli, 2022) .

Table 2. Effect of cultivars on chemical properties of Niger seed.

Cultivars	Oil	Protein	Ash	Fiber	Carbohydrate	Moisture
Benglanuglue	33.34 a	22.82ab	3.03b	20.10b	11.99b	4.42a
Karal	32.73 b	21.32b	3.36b	21.40a	13.13a	4.02a
Animax	32.95 b	23.45a	4.45a	20.85b	8.08c	4.03a

At 5% of DMRT, means with the same litters for each factor and interaction do not differ significantly

Statistical analysis demonstrated a significant genotypic influence on carbohydrate content. The highest content of carbohydrate was 13.13% obtained by Karal cultivar. However, the lower content of carbohydrate was 8.08% obtained by Animax cultivar (Hassan, 2023). The observed carbohydrate content in the current study aligns with previous reports, which have generally indicated higher carbohydrate levels in other Niger accessions (Syume and Chandravanshi, 2015). The elevated carbohydrate content within Niger seeds significantly enhances their nutritional profile, and establishing them as a valuable energy source for human consumption thereby improving their organoleptic properties (Kaur et al., 2013)

Moisture content, as characterized by (Pomeranz and Meloan 1994), quantifies the mass of water and volatile compounds eliminated during desiccation. In this study, the moisture content of Niger seed samples ranged from 4.02% to 4.42%. The 'Benglanuglue' cultivar exhibited the highest moisture content, while the 'Karal' cultivar displayed the lowest. The observed moisture content values in this study align with previously reported ranges for different Niger accessions (Jagtap et al., 2014 and Fatima et al., 2015). Moisture content is a critical factor influencing seed longevity, with lower moisture levels generally associated with improved storage stability (Sarker and Ercisli, 2022). While low moisture content is generally desirable for food processing and long-term storage (Uyoh et al., 2013), the moisture content of the Niger cultivars evaluated in this study fell within the acceptable range (>12%) for long-term preservation, as outlined by (Mbanjo et al. 2020) .

The fatty acid profile of Niger seed oil is characterized by the presence of linoleic acid (C18:2), linolenic acid, oleic acid, palmitic acid, and stearic acid. Notably, Linoleic acid (C18:2) was identified as the primary unsaturated fatty acid among all Niger seed varieties analyzed in this investigation, representing a significant portion of the total fatty acid profile, ranging from 18.89% to 24.16%. (Table 3). Linolenic acid (C18:3) ranked as the second most prevalent unsaturated fatty acid, comprising 16.20% to 18.43% of the total fatty acid composition .

Palmitic acid (C16:0) and stearic acid (C18:0) were identified as the predominant saturated fatty acids, contributing (15.83 to 16.58) % and (4.82 to 4.98) % to the overall fatty acid profile, respectively. Significant genotypic variability in fatty acid composition was observed among the Niger seed oil samples, with the exception of stearic acid content (Table 3). The 'Benglanuglue' cultivar exhibited the highest concentrations of oleic, linolenic, palmitic, and stearic acids. The 'Animax' cultivar displayed the highest proportion of linoleic acid, reaching 24.16 %. The fatty acid ratios determined in this study for the various cultivars were lower than those reported in previous investigations by (Deme et al. 2021).

There are differences in fatty acid content in our result compared with what had been obtained from Niger seeds because of these reasons: Several factors contribute to the variation in plant oil content and composition including temperature, cultivar, age and maturity and planting dates to varying extents (Greve et al., 1992) .

Moreover, the specific fatty acid composition exerts a profound impact on both the sensory attributes (e.g., flavor) and the overall physicochemical properties of the extracted oil (Aşkın, 2018). Variations in fatty acid profiles are observed across different plant species, cultivars, and are further influenced by environmental growing conditions (Sabzalian et al., 2008 and Kostik et al., 2013). The 'Karal' cultivar exhibited the lowest palmitic acid content relative to other evaluated cultivars. This characteristic is highly desirable for culinary applications, as a reduced intake of saturated fatty acids, such as palmitic acid, is strongly linked to decreased blood cholesterol levels .

Table 3. Effect of cultivars on some fatty acids properties of Niger seed oil.

Cultivars	Oleic	Linoleic	Linolenic	Palmitic	Steric
Benglanuglue	8.12a	16.89c	18.43a	16.58a	4.98a
Karal	7.78a	20.45b	16.43b	15.83b	4.88a
Animax	2.08b	24.16a	16.20b	16.37ab	4.82a

At 5% of DMRT, means with the same litters for each factor and interaction do not differ significantly.

CONCLUSION:

Based on the experiment, we conclude that there are significant differences between the selected Niger cultivars in the germination characters, anatomy, phenological, physical and chemical characteristics, the Animax and Benglanuglue have highest rates of the essential fatty acid linoleic and linolenic acid with highest oil% and most of the studied characteristics.

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