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A TAXONOMIC ANALYSIS OF THE SUDANESE MEDICINAL PLANT, *VACHELLIA NILOTICA* L. (SYN*: ACACIA NILOTICA* L.) BASED ON PODS, SEEDS, AND VEGETATIVE TRAITS

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Abstract

Vachellia nilotica L. (*Syn: Acacia nilotica*) has puzzled *taxonomic* history and nomenclature. This study aimed to update the taxonomy of this species in the Sudan by identifying its subspecies and morphological differences among them. The study was carried out during the period from March 2020 to September 2022. The included collection sites were Riverine Forest s, seasonal streams, seasonal rivers, natural reserved forests, and natural areas out of reserved forests. The species phenology (vegetative growth, flowering, fruiting, and seeding time) was considered to facilitate delineation between its sub-taxa in each site during field surveys and data collection, which was done in 100 randomly selected circular plots (1000 m^2). Morphological characterization of the vegetative parts (16 traits) and pods and seeds (14 traits) was studied on 100 trees selected randomly for each identified sub-taxa in each site. The results indicated for the first time a new group intermediate in all studied morphological characteristics between the subspecies *nilotica* and *tomentosa* which were also identified in this study beside the subspecies *adstringens*. Furthermore, analysis of variance showed significant (P = 0.0001) variation among them in vegetative, pod, and seed characteristics. Pod shape, color, and texture; seed shape and size and number of pinnae per leaf were key features to distinguish between the subspecies and the new group. This study gave evidence for the first time for a new morphological group of *Vachellia nilotica* L. (Syn: *Acacia nilotica*) with intermediate characteristics of pods, seeds, and leaves and habit between subspecies *nilotica* and *tomentosa* at the mature stage, indicating potential hybridization among the subspecies.

Key words: *Vachellia nilotica;* Fabaceae; Morphology, Phenotyping, Taxonomy, Sudan, Species, Subspecies, Life on land.

Introduction

Vachellia nilotica Hunter & Mab (=*Acacia nilotica* (L.) Willd. ex Del) of the family Fabaceae, sub-family Mimosoideae, tribe Acacieae is commonly known as babul, kikar, or Indian gum Arabic tree (Caesariantika *et al*., 2011). It has been recognized worldwide as a multipurpose leguminous tree species (Kaur *et al*., 2005; Solomon-Wisdom & Shittu, 2010; Elzaki & Gang, 2019). The species has a wide range of natural distribution in tropical and subtropical areas from Egypt and Mauritania southwards to southern Africa and Asia eastward to the Indian subcontinent (Caesariantika *et al*., 2011; Solomon-Wisdom and Shittu 2010; Mahmood *et al*., 2005). Moreover, it is valuable mainly for timber and fuel wood production (Elzaki & Gang, 2019), tannin production (Haroun *et al*., 2009; Elkhalifa *et al*., 2005 and Banso, 2009). Additionally, it has medicinal attributes (Banso 2009; Abdallah, 2016) and plays a role in Agroforestry (Bargali *et al*., 2009) and environmental conservation (Warrag *et al*., 2002).

According to the modern molecular-based system of flowering plant taxonomy (angiosperms), the accepted name is *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Syn: *Acacia nilotica* (L.) Willd. ex Delile (Maslin *et al*., 2003; Kyalangalilwa *et al*., 2013). This species is a polyploidy complex within the genus *Acacia,* which is characterized by the base chromosome number "x = 13" (*Bennett*, 2000).

The diversity of sites and climatic conditions under which this species grows led to the evolution of an extremely variable species a puzzled taxonomic history and nomenclature. Thus, its taxonomy worldwide is recognized by nine subspecies; among these, the subspecies *indica, cupressiformis,* and *hemispherica* are native to India and Pakistan, subspecies *hemispherica* is endemic to Pakistan (Mahmood *et al*., 2005) and the subspecies *nilotica, tomentosa, leiocarpa, subalata, kraussiana,* and *adstringens* are from Africa (Chhillar *et al*., 2002).

The main characteristic currently used for subspecies identification is the tree-canopy habit that is evergreen to semi-evergreen with an ovoid crown in the subspecies *nilotica*, *tomentosa,* and *indica* to deciduous with a spreading crown in the subspecies *adstringens*, *subalata*, *leiocarpa,* and *kraussiana*. On the other hand, subspecies *cupressiformis* and *hemispherica* are different and have a narrow-erect and bushy crown, respectively (Ali & Qaiser, 1992; Chhillar *et al*., 2002).

Branchlet pilosity is either glabrous, glabrescent, puberulous, pubescent, or tomentose and mature pod characteristics in the term of shape, margin shape, and degree of the pubescence are either "necklace-like, narrowly and regularly constricted between seeds with different degrees of pubescence and colour", as in the subspecies *tomentosa*, *nilotica*, *indica*, and *cupressiformis*, or “not necklace-like with straight or crenate margins and different degree of pubescence and colour” as in the subspecies *leiocarpa*, *subalata*, *kraussiana*, *adstringens*, and *hemispherica* (Chhillar *et al*., 2002; Mahmood *et al*., 2005). In Africa, wooded grassland, dry scrub forests, and savanna are the ecological preferences for subspecies *subalata*, *leiocarpa*, and *adstringens*, while riverine habitats and seasonally flooded areas are preferable for subspecies *nilotica* and *tomentosa*. Further, subspecies *kraussiana* prefers dry grasslands and savannas, especially on compacted sandy loam, shallow granite, or clay soils along drainages and rivers, but away from flooding (Mahmood *et al*., 2005). On the Indian subcontinent, subspecies *indica* forms low-altitude dry forests frequently on alluvium soils; subspecies *hemispherica* it is a narrow endemic, restricted to a small coastal belt and subspecies *cupressiformis* has similar preferences to subspecies *indica*, though it is less resilient to weed competition (Mahmood *et al*., 2005).

In the Sudan, *Vachellia nilotica* (Syn: *Acacia nilotica*) is commonly known by “Sunt or Grad” has a long history of management as a forestry species since the establishment of the plantations in 1935 (Mahgoub, 2008). Sudan’s *Vachellia nilotica* is represented by four subspecies with wide range of distribution, among them the subspecies *nilotica* and *tomentosa* are found in the flooded sites along the River Nile bank and its tributaries, while the subspecies *adstringens* is encountered in the dry areas on gravely or alluvial soils in Kordofan and Darfur, mainly along seasonal rivers and valleys; and the subspecies *subulata* has been stated to occur in savanna grassland forming thicket with *Commiphora africana* in northern and southeast Equatoria (Kordofani & Ingrouille, 1991; Alaklabi, 2015). Furthermore, Sahni (Alaklabi, 2015) stated the presence of subspecies *indica* as an exotic species of pods with appearance and characteristics similar to subspecies *tomentosa*, this occurrence needs more confirmation.

It remains unclear in *Vachellia nilotica* taxonomy whether to classify it as a subspecies or a species. Elberse et al. stated that species with a wide geographic distribution and diverse habitats can show extremely high morphological variability (Elberse *et al*., 2003).

Therefore, AN has a wide ecological amplitude and variety of growing habitats (Mahmood *et al*., 2005). Remarkable variations were stated according to the subspecies in morphological expressions for leaf, stipular spine, pod (Mahmood *et al*., 2005), and seed (Chhillar *et al*., 2002). Recently, the identification of AN subspecies depends on Brenan’s key, which is an old and limited since it considered only the morphological variability of pod traits with no or few considerations for the variability of other morphological characteristics (Nations, 2023). However, Wardill *et al*., (2005) stated a serious difficulty in subspecies recognition, particularly when pods are absent (Wardill *et al*., 2005). This, with no doubt makes the Brenan’s key very tenuous for two reasons. Firstly, the characters used can exhibit a wide range of diversity within individuals, populations, and subspecies, making subspecies difficult to designate (especially without seed pods); secondly, hybridization between particular subspecies occurs commonly and produces many morphotypes, blurring their distinctions (Wardill *et al*., 2005). Thus, an interpretation of data relative to *Vachellia nilotica* may raise problems if we do not clarify the subspecies about which we speak. Morphological traits were among the earliest markers used in plant taxonomy, germplasm management, and breeding programs, but they have several limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Beyene *et al*., 2005) particularly the quantitative traits (Govindaraj *et al*., 2015).

The overall objective of the present study was to update the taxonomy of *Vachellia nilotica* and understand the level of morphological diversity and similarity among its subspecies in the Sudan by analyzing the quantitative and qualitative measures of pods, seeds, and vegetative parts (leaves and stipular spines traits).

Material and Methods

Sampling procedure: The study was conducted during the period from March 2020 to September 2022. Thirteen sites representing both artificially managed and/or natural plantations of AN were selected for sampling and described for location within the agro-ecological zones of Sudan, general soil condition, topography, and rainfalls (Table 1). In total, 100 circular sample plots (1000 m^2) were allocated randomly in each site by using handle GPS and surveyed during seasons of the vegetative growth (June-October), flowering (July-September), and fruiting and seeding (March-May) of the species in Sudan.

Species identification: Tree individuals in each site were identified to subspecies and/or new group of AN based on morphological characteristics of pods, branches, and branchlets as described in Brenan's (Nations 2023) key to subspecies (Table 2). Then in each site and for each identified sub-taxa, 100 trees (dbh 25-35 cm) were selected randomly in space not less than 100 m apart to reduce the relatedness of the mother trees (Abdelkheir *et al*., 2011) for material and data collection. For morphological characterization of pods and seeds, fifty ripe and healthy pods were harvested from the crown (top, bottom, and lower) of each single tree and kept separately in clean polythene bags after measuring its length in cm, width, constriction width, using 150 mm-digital vernier caliper (Modle: GT-DC-01, Globaltronics GmbH & Co. KG) and the number of seeds per pod was also counted.

Table 1. The study sites, location coordinates distribution within the agro-ecological zone of Sudan and general habitat conditions in Sudan.

Study site (Code)	Coordinate	Location within agro-ecological zone of Sudan	General habitat conditions
Shambat open farms (**Sha**)	15° 39' N, 32° 30' E	Semi-desert: *Acacia tortilis – Maerua crassifolia* Desert Scrub; mean annual rainfalls 75 - 300 mm/year	Private farms, seasonal streams, and along the River Nile banks
Alsunt Riverine Forest (**Alsu**)	15°35' N, 32° 30' E		Riverine Forest reserve, on White Nile River, loamy soil
Alsnait Riverine Forest (**Alst**)	14 ° 44 'N, 33° 22' E	Semi-desert: Grassland on clay soils, with dominance of *Acacia mellifera*; mean annual rainfalls 400 mm	Riverine Forest reserve, on Blue Nile River, loamy soil
Aldenaigila Riverine Forest (**Alden**)	13° 53' N, 32° 37' E		Riverine Forest reserve, on Blue Nile River, clay soil
Abu Geili Riverine Forest (**AbG**)	13° 34' N, 32° 35' E.	Low rainfall woodland savanna on clay soils; mean annual rainfalls vary from isohyets 400-600 mm	Riverine Forest reserve, on Blue Nile River, clay-loam soil
Allmbwa Riverine Forest (**Alm**)	13° 00' N, 33° 59' E		Riverine Forest reserve, on Blue Nile River, clay-loam soil
Um Zibil Riverine Forest (**UmZ**)	13° 23' N, 34° 38' E	Low rainfall woodland savanna on clay soils; mean annual rainfalls vary from isohyets 400-600 mm	River forest reserve, Al Rahd seasonal River, clay soil
Aldeisa Riverine Forest (**Alde**)	12° 03' N, 34° 18' E.	Low rainfall woodland savanna on clay soils, mean annual rainfalls vary from isohyets 600-800 mm	Riverine Forest reserve, west of the Blue Nile River, clay-loam soil
Sawlail Riverine Forest (**Saw**)	12° 19' N, 34° 21' E		Riverine Forest reserve, east of the Blue Nile River, clay-loam soil.
Klikis Natural Forest (**Kli**)	12° 58' N, 32° 53' E	Low rainfall woodland savanna on sandy soils; mean annual rainfalls vary from isohyets 400-600 mm	Riverine Forest reserve, White Nile River, sandy-loam soil
El Ain Natural Forest (**Ela**)	12° 59' N, 30° 20' E	Semi-desert: Grassland on sandy soils, mean annual rainfalls 150-450 mm	Natural Forest reserve, mountainous slope, sandy-clay soil
Nabak Natural Forest (**Nab**)	12° 31' N, 29° 55' E	Low rainfall savanna on sandy soils; mean annual rainfalls range between 300-500 mm southwards	Natural Forest reserve, sand dune slope, sandy-clay soil
Kadogli open lands (**Kadg**)	11° 01' N, 29° 42' E & 11° 02' N, 30° 04' E	Low rainfall savanna on sandy soils; mean annual rainfalls range between 600-800 mm southwards	Natural Forest area, mountainous slope, sandy-clay soil

Table 2. Used Pod and branchlet traits to identify *Acacia nilotica* subspecies in the field.

Subspecies	***Acacia nilotica* subspecies morphology based on Brenan (1983) and El Amin (1990)**	
	Pod characteristic	**Branch & branchlet characteristics**
Nilotica	Pods necklace-like, glabrous, or almost so, narrowly, and regularly constricted between the seeds	Young branchlets are glabrous to shortly puberulous and old branches brown to brownish in color
Tomentosa	Pods are necklace-like, grey to white-tomentellous or almost so, narrowly, and regularly constricted between the seeds	Young branchlets are densely white-tomentose and the old one is grayish to dark grey in color
Adstringens	Pods not necklace-like, densely, and persistently tomentose, with distinctly but often irregularly crenate margins	Young and old branchlets are densely tomentose

Pods were evaluated qualitatively for their color and surface texture. The seeds were extracted manually from evaluated pods, cleaned from debris, and then kept separately for each single tree in polythene bags. One hundred seeds were taken randomly from each bag and weighed to determine the weight of 100 seeds using a digital sensitive balance (FA series 100g/0.1mg) and the number of seeds per kilogram was determined (100 seeds X 1000 (g) /weight of 100 seeds (g)). Then 50% of the weighted seeds (50 seeds/single tree) were measured in cm by an electronic vernier caliper for length, width, and thickness. Afterward they were qualitatively evaluated for their surface texture, color, and areole shape. Furthermore, the seed shape index (seed length/seed width) was calculated and used to evaluate the seed shape either Globose to globose-oval (1–1.25), oblong (1.30–3.3), or elongated (5–10). The pods and seeds were photographed for each identified group by using a Nikon digital camera (Nikon COOLPIX S3100, NIKON CORP., JAPAN). Morphological characterization of vegetative parts (foliar and stipular spines traits) was made in 15 mature branches (20 cm long), which were harvested from the crown (bottom, middle and possibly top) of each single tree and quantitatively evaluated for the number of compound leaves (in pairs), leaf length (cm), petiole length (cm), number of pinnae's (in pairs) per compound leaves, pinnae length (cm), pinnae width (cm), number of leaflets (in pairs) per pinnae, leaflet length (cm), leaflets width (cm), number of stipular spines (in pairs) per 20 cm branch and stipular spine length (cm). The qualitative evaluation was for leaflet color, leaflet shape, branchlet color and texture, and stipular spine color. The tree habit and the texture and color of the main stem of mature trees were also determined. All quantitative measurements and photographs of the trees` vegetative parts were performed by the previously mentioned digital vernier caliper and Nikon digital camera, respectively.

Table 3. The presence and absence of the subspecies of *Vachellia nilotica* and/or new group in each of the study sites.

Study sites	*Acacia nilotica* sub-taxa			
	Nilotica	*Tomentosa*	New group	*Adstringens*
Aldeisa (Alde)	1	1	1*	0
Sawlail (Saw)	1	1	1	0
Allmbua (Alm)	1	1	1	0
Abu Geili (AbG)	1	1	0	0
Aldeneigila (Alden)	1	1	1*	0
Alsnait (Alst)	1	1	1	0
Um Zibil (UmZ)	1	1	0	0
Alsunt (Alsu)	1	1	0	0
Shambat (Sha)	1	1	1	0
Klikis (Kli)	0	1	0	0
El Ain (Ela)	1	1	1*	1
Nabak (Nab)	0	0	0	1
Kadogli (Kadg)	0	0	0	1
Total present	10	11	7	3
Frequency (%)	76.92	84.62	53.85	23.08
Relative frequency (%)	32.26	35.48	22.58	9.68

(1): Present and sampled; (0): Absent; (1*): Present but not sampled

Statistical analysis

The morphological data of mature trees (pods, seeds, leaves, and stipular spines) were subjected to analysis of variance (ANOVA) to test the significance of variation among and within *Acacia nilotica* sub-taxa by using GLM procedure in STATSTICA software (SAS Analytics, 2023) and means were calculated using Duncan's Multiple Range Test. Spearman's similarity or correlation coefficient (Spearman, 2010) was calculated among identified genotypes of *Acacia nilotica* (subsp. *nilotica*, *tomentosa*, *adstringens,* and new group) from the morphological data and matrices which were constructed using the statistics software package PAST (PAleontological Statistics) version 3.01 (Palaeo-electronica, 2023). The obtained similarity matrices were then clustered in a phenogram (dendrogram) using the Unweighted Pair-Group Method with the Arithmetic Mean (UPGMA) Method (Sani & Maiwada, 2018).

Results

Identification of *Vachellia nilotica* sub-taxa and their occurrence frequency (%) in the study area: In this study, four sub-taxa of *Vachellia nilotica* (L.) Hunter & Mab. were identified across the study area (13 variable sites) based on morphological variation of pods, seeds, and vegetative (branches, branchlets, leaves, and stipular spines) characteristics (Table 3).

The subspecies *nilotica*, subspecies *tomentosa*, subspecies *adstringens,* and the new group of individuals showed intermediate morphological characteristics of pods, seeds, and vegetative parts between the subspecies *nilotica* and *tomentosa*. The new group was identified in seven sites giving a relative frequency of 22.58%, among which Aldeisa, Sawlail, Allmbua, Aldeneigila, and Alsnait were Riverine Forest reserve; Shambat is a natural area; and El Ain is a natural forest reserve, as a mixed population with subspecies *nilotica* (32.26 % relative frequency) and *tomentosa* (35.48% relative frequency).

The subspecies *adstringens* was of less relative frequency (9.68%) since it occurred only at Al Ain and Nabak (natural forest reserve) and Kadogli (natural area). The subspecies *nilotica* and *tomentosa* were usually in a mixed population except in Klikis, Nabak, and Kadogli where only the subspecies *tomentosa* and *adstringens* were identified in pure stands.

Quantitative and qualitative morphological variations in fruit and seed traits among *Vachellia nilotica* L. (Syn: *Acacia nilotica*) sub-taxa: Significant differences (p=0.0001) were recorded among the identified sub-taxa of AN in the morphological characteristics of pods (Table 4) and seeds (Table 5).

The subspecies *nilotica* and *tomentosa* had almost similar pods that were the narrowest necklace-like with an average width of 1.33 ± 0.15 cm for the subspecies *nilotica* and 1.35 ± 0.15 cm for the subspecies *tomentosa*. In the subspecies *nilotica*, the overall average width of the constriction between the seeds was 0.25 ± 0.11 cm, while it was 0.39 ± 0.13 cm in the subspecies *tomentosa*. The pods of the subspecies *nilotica* were dark-brown to black, glabrous, glossy, and ridged with an overall average length of 13.45 ± 4.87 cm and an overall average number of 10.22 ± 2.04 seeds per pod (Fig. 1a).

The subspecies *tomentosa* pods were dark grey-greyish in colour, pubescent-tomentose, ridged (Fig. 1b) that were similar to those of subspecies *adstringens* with great variation in overall average length that was 12.49 ± 2.90 cm and overall average number of seeds per pod of 9.51 ± 1.87. The new group was very distinct from the other subspecies by its pale brown-brownish, glabrous, glossy, ridged pods that were necklace-like (Fig. 1c), intermediate in average width that was 1.67 ± 0.14 cm and an irregular constriction between the seeds with an average width of 0.45 ± 0.14 cm. It had the longest and the straightest pods with an average length of 14.92 ± 7.45 cm, as well as the highest number of seeds per pod with an average of 11.20 ± 1.54. The subspecies *adstringens* had distinct pods that were grey-greyish, pubescent-tomentose, redged falcate to slightly straight, very wide in dimensions with an average width of 1.80 ± 0.14 cm; a constriction between the seeds was also the widest with an average width of 1.59 ± 0.7 cm giving not necklace-like pods with crenate or entire margin (Fig. 1d, 1e and 1f). Pods of the subspecies were the shortest than those of other genotypes with an average length of 11.37 ± 2.27 cm and consequently the lowest number of seeds per pod with an average of 9.27 ± 1.61. The seeds of the subspecies *nilotica* were oval, dark brown, and shiny (Fig. 2a) less than other subspecies in an overall average length, width, and thickness that was 0.76 ± 0.06 cm, 0.69 ± 0.08 cm and 0.34 ± 0.05 cm, respectively (Table 5).

The subspecies *tomentosa* (Fig. 2b) and the new group (Fig. 2c) had similar oblong, smooth, and dark brown-glossy seeds, with slightly difference in overall average length, width and thickness that were 0.78 ± 0.07, 0.59 ± 0.06, and 0.35 ± 0.05 cm, respectively for subsp. *tomntosa* and 0.82 ± 0.07, 0.58 ± 0.03, and 0.35 ± 0.05 cm for the new group (Table 5). The subspecies *adstringens* had very large, globose-oblong, and dark brown-glossy seeds (Fig. 2d, 2e and 2f) with an overall average length, width, and thickness of 0.91 ± 0.08, 0.74 ± 0.06, and 0.42 ± 0.04 cm, respectively (Table 5).

Table 4. Pod morphological variation (quantitative and qualitative traits) among the subspecies and the new group of *Vachellia nilotica* in Sudan.

Characters	Genotypes				Pr > F
	nilotica	***New group***	***tomentosa***	***adstringens***	
Pod length (cm)	13.45b (± 4.87)	14.92a (± 7.45)	12.49c (± 2.90)	11.37d (± 2.27)	0.0001
Pod width (cm)	1.33d (± 0.15)	1.67b (± 0.14)	1.35c (± 0.15)	1.80a (± 0.14)	0.0001
Pod constriction width (cm)	0.25d (± 0.11)	0.45b (± 0.14)	0.39c (± 0.13)	1.59a (± 0.70)	0.0001
Number of seeds per pod	10.22b (± 2.04)	11.20a (± 1.54)	9.51c (± 1.87)	9.27d (± 1.61)	0.0001
Pod colour	Dark brown or black-shiny	Pale brown to brownish	Grey to grayish	Grey to greyish	-
Pod surface texture	Glabrous-Shiny	Fearly glabrous-Shiny	Pubescent-tomentose	Pubescent-tomentose	-

Means sharing the same letter in the same column do not differ significantly at P= 0.05 according to Duncan's Multiple Range Test; Means ± Standard deviation

Table 5. Morphological variation in seed traits (quantitative and qualitative) among the subspecies and the new group of *Vachellia nilotica* in the Sudan.

Characters	Identified groups of *Vachellia nilotica*				Pr > F
	Subsp. *nilotica*	New group	Subsp. *tomentosa*	Subsp. *adstringens*	
Seed length (cm)	0.76d (± 0.06)	0.82b (± 0.07)	0.78c (± 0.07)	0.91a (± 0.08)	0.0001
Seed width (cm)	0.69b (± 0.08)	0.58c (± 0.03)	0.59c (± 0.06)	0.74a (± 0.06)	0.0001
Seed thickness (cm)	0.34c (± 0.05)	0.35b (± 0.05)	0.35b (± 0.05)	0.42a (± 0.04)	0.0001
Seed size (cm)	0.60b	0.58c	0.57d	0. 69a	0.0001
Weight of 100-seeds (g)	11.49c (± 0.83)	11.79b (± 0.74)	11.74b (± 1.19)	23.31a (± 2.36)	0.0001
Number of seeds per Kilogram	8741a (± 585.76)	8511b (± 494.21)	8588b (± 757.51)	4332c (± 414.94)	0.0001
Seed shape index	1.10b (Globose)	1.34a (Oblong)	1.32a (Oblong)	1.22b (Globose)	0.0001
Seed colour	Dark brown, shiny	Brownish, shiny	Dark brown, shiny	Dark brown, shiny	-
Seed surface texture	Smooth	Smooth	Smooth	Smooth	-
Areole shape	Oval	Oblong	Oblong	Ovate & Oblong	-

Means sharing the same letter in the same column do not differ significantly at P= 0.05 according to Duncan's Multiple Range Test; Means ± Standard deviation

An opposite trend was observed among all the studied subspecies and the group in the overall average weight of 100-seeds and overall average number of seeds per kilogram. The subspecies *adstringens* had the highest average weight of 100-seeds (23.31g), as well as the lowest average number of seeds per kilogram (4332 seeds) opposite to the subspecies *nilotica,* which showed the lowest average weight of 100 seeds (11.49 g) and the highest average number of seeds per kilogram (8741 seeds) (Table 5). The new group and the subspecies *tomentosa* were very close to each other in the overall average weight of the 100 seeds (g) that was respectively 11.79 g and 11.74 g and also in the overall average number of seeds per kilogram that was 8511 (seeds) and 8588 (seeds), respectively. Furthermore, great variation was observed among the three and the new group in the seed's areole shape, which was oval in both subspecies *nilotica* and *adstringens* and oblong in the subspecies *tomentosa* and the new group.

Quantitative and qualitative morphological variations in vegetative parts traits among *Vachellia nilotica* (*Acacia nilotica*) sub-taxa: There were significant (P=0.0001) differences between identified AN sub-taxa vegetative part morphological trait (Table 6). The subspecies *tomentosa, adstringens,* and the new group had the highest average number of leaves per 20 cm branch of 9.16 ± 2.43, 9.16 ± 2.21, and 9.02 ± 2.17 cm, respectively, while the lowest average was 8.02 ± 2.47 cm scored in the subspecies *nilotica*. Furthermore, the new group had the longest leaves with an average of 10.98 ± 1.66 cm followed by the subspecies *tomentosa* (8.33 ± 1.44 cm) and *nilotica* (6.89 ± 1.12 cm), while the subspecies *adstringens* had the shortest leaves (5.25 ± 0.65 cm). The average number of pinnae pairs per compound leaf showed a high taxonomic value in the delineation between the subspecies and the new group of *Acacia nilotica* (Table 6). The average number of pinnae pairs per compound leaf was in the following order: 9.85 ± 0.61, 8.19 ± 0.72, 6.68 ± 0.75, and 4.64 ± 0.49 for the new group, the subspecies *tomentosa*, the subspecies *nilotica,* and the subspecies *adstringens,* respectively. The average pinnae length in the new group was 3.58 ± 0.41 cm, as significantly the highest average, followed by 3.38 ± 0.49 cm in the subspecies *tomentosa* and 3.20 ± 0.37 cm in the subspecies *adstringens*, while it was 2.82 ± 0.41 cm, as the lowest average, in the subspecies *nilotica*.

The results showed that a similar trend could be observed between the average pinnae width (cm) and the average pinnae length (cm) among the three identified subspecies and the new group of *Vachellia? nilotica*. The new group had, in average, the longest leaflets of 0.47 ± 0.08 cm and consequently the widest pinnae of 0.96 ± 0.17 cm, followed by the subspecies *tomentosa* that had an average leaflet length of 0.42 ± 0.08 cm and average pinnae's width of 0.84 ± 0.16 cm. The shortest leaflets and the narrowest pinnae were found in subspecies *nilotica* (0.78 ± 0.16 cm and 0.78 ± 0.16 cm) and *adstringens* (0.38 ± 0.06 cm and 0.77 ± 0.11cm). The average number of leaflets per pinnae can be used to distinguish between the three identified subspecies and the new group when other characters like the pods and seeds are not available. The average number of leaflets in pairs was 24.33 ± 4.60 for the subspecies *nilotica*, 29.93 ± 2.33 for the new group, 27.31 ± 4.23 for the subspecies *tomentosa*, and 18.52 ± 2.40 for the subspecies *adstringens*. Moreover, no significant difference in the leaflets` width was observed for the subspecies *nilotica* and *tomentosa,* which had an average width of 0.13 ± 0.06 cm but dark green leaflets (Fig. 3a) and 0.13 ± 0.02 cm pale green leaflets (Fig. 3b), respectively.

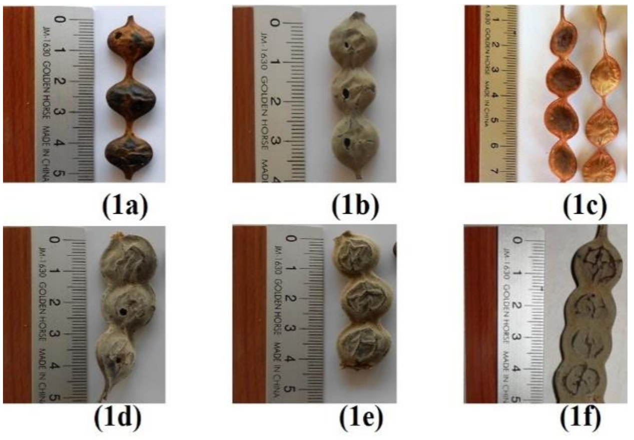

Fig. 1. Typical pods shape, color, texture, and constriction pattern of the subspecies *nilotica* (**1a**), *tomentosa* (**1b**), the new group (**1c**), and the subspecies *adstringens* (**1d**, **1e**, and **1f**).

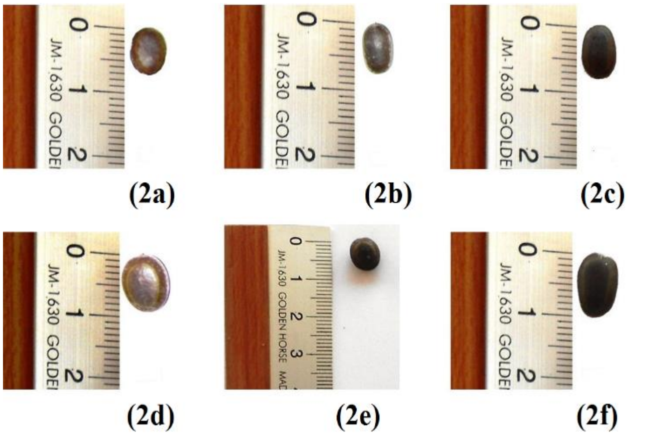

Fig. 2. Typical seed shape, colour, and texture of the subspecies *nilotica* (**2a**), *tomentosa* (**2b**), the new group (**2c**), and the subspecies *adstringens* (**2d**, **2e**, and **2f**).

On the other hand, the new group had also pale green leaflets (Fig. 3c) but with an average width of 0.14 ± 0.07 cm less than the subspecies *adstringens* which is distinct from other sub-taxa by its widest pale green leaflets (Fig. 3d, 3e and 3f) with an average of 0.18 ± 0.11 cm.

The spines were white in colour for the subspecies *nilotica* (Fig. 3a), *tomentosa* (Fig. 3b), and the new group (Fig. 3c) but they were pale brown in colour and sometimes white for the subspecies *adstringens* (Fig. 3d, 3e and 3f). However, the sub-taxa showed variation in the average number of spines (pairs) per 20-cm branch that was in the following order; 8.56 ± 2.76 and 8.79 ± 2.03 as the lowest and 9.81 ± 2.86 and 9.92 ± 2.62 as significantly the highest, for the subspecies *nilotica, adstringens,* the new group, and *tomentosa*, respectively (Table 6). Furthermore, the average stipular spine length (cm) was 5.62 ± 3.14, 4.60 ± 1.43, 4.39 ± 2.02, and 4.11 ± 2.50 for the subspecies *adstringens*, the new group, the subspecies *tomentosa*, and *nilotica* (Table 6).

The outer bark of the main stem overlapped among the studied sub-taxa at maturity. It was black-dark brown, slightly longitudinally fissured in subspecies *nilotica* (Fig. 4a); the bark was dark grey-brownish in subspecies *tomentosa* (Fig. 4b) closer to the new group, where the bark was dark brown-brownish deeply fissured, longitudinally and spirally (Fig. 4c). However, the subspecies *adstringens* had bark characteristics like subspecies *tomentosa* (Fig. 4d and 4e) when being in tree habit, while fissured brownish bark was also observable within the subspecies *adstringens* (Fig. 4f).

Table 6. Results of Duncan's Multiple Range Test (DMRT) for vegetative traits of mature trees among the subspecies and the new group of *Acacia nilotica* in Sudan.

Characters	Identified groups of *Vachellia nilotica*				Pr > F
	subsp. nilotica	**New group**	**subsp. *tomentosa***	**subsp. *adstringens***	
Number of leaves per 20-cm branch	8.02b (± 2.47)	9.02a (± 2.17)	9.16a (± 2.43)	9.16a (± 2.21)	0.0001
Leaf length (cm)	6.89c (± 1.12)	10.98a (± 1.66)	8.33b (± 1.44)	5.25d (± 0.65)	0.0001
Petiole length (cm)	1.66b (± 0.37)	1.36d (± 0.27)	1.44c (± 0.23)	1.70a (± 0.17)	0.0001
Number of pinnae (pairs) per leaf	6.68c (± 0.75)	9.85a (± 0.61)	8.19b (± 0.72)	4.64d (± 0.49)	0.0001
Pinnae length (cm)	2.82d (± 0.41)	3.58a (± 0.41)	3.38b (± 0.49)	3.20c (± 0.37)	0.0001
Pinnae width (cm)	0.78c (± 0.16)	0.96a (± 0.17)	0.84b (± 0.16)	0.77c (± 0.11)	0.0001
Number of leaflets (pairs) per pinnae	24.33c (± 4.60)	29.93a (± 2.33)	27.31b (± 4.23)	18.52d (± 2.40)	0.0001
Leaflet length (cm)	0.39c (± 0.08)	0.47a (± 0.08)	0.42b (± 0.08)	0.38c (± 0.06)	0.0001
Leaflet width (cm)	0.13c (± 0.06)	0.14b (± 0.07)	0.13c (± 0.02)	0.18a (± 0.11)	0.0001
Number of stipular spines per 20-cm branch	8.56b (± 2.76)	9.81a (± 2.86)	9.92a (± 2.62)	8.79b (± 2.03)	0.0001
Stipular spine length (cm)	4.11c (± 2.50)	4.60b (± 1.43)	4.39b (± 2.02)	5.62a (± 3.14)	0.0001
Mature branches colour	Dark brown	Brownish	Greyish- brownish	Greyish- brownish	
Mature branches texture	Glabrous	Fairly tomentose	Pubescent-tomentose	Pubescent-tomentose	-
Leaf and pinnae colour	Dark green	Pale green	Pale green	Pale green	-
Bark colour of main stem	Dark brown-brown	Brown	Greyish-brown	Greyish-brown	-
Bark texture	Deeply, longitudinally fissured	Slightly, longitudinally fissured	Deeply, longitudinally fissured	Deeply, longitudinally fissured & rough	-
Stipular spine colour	White	While	White	Pale brown	-

Means sharing the same letter in the same row do not differ significantly at P= 0.05 according to Duncan's Multiple Range Test; M ± SD

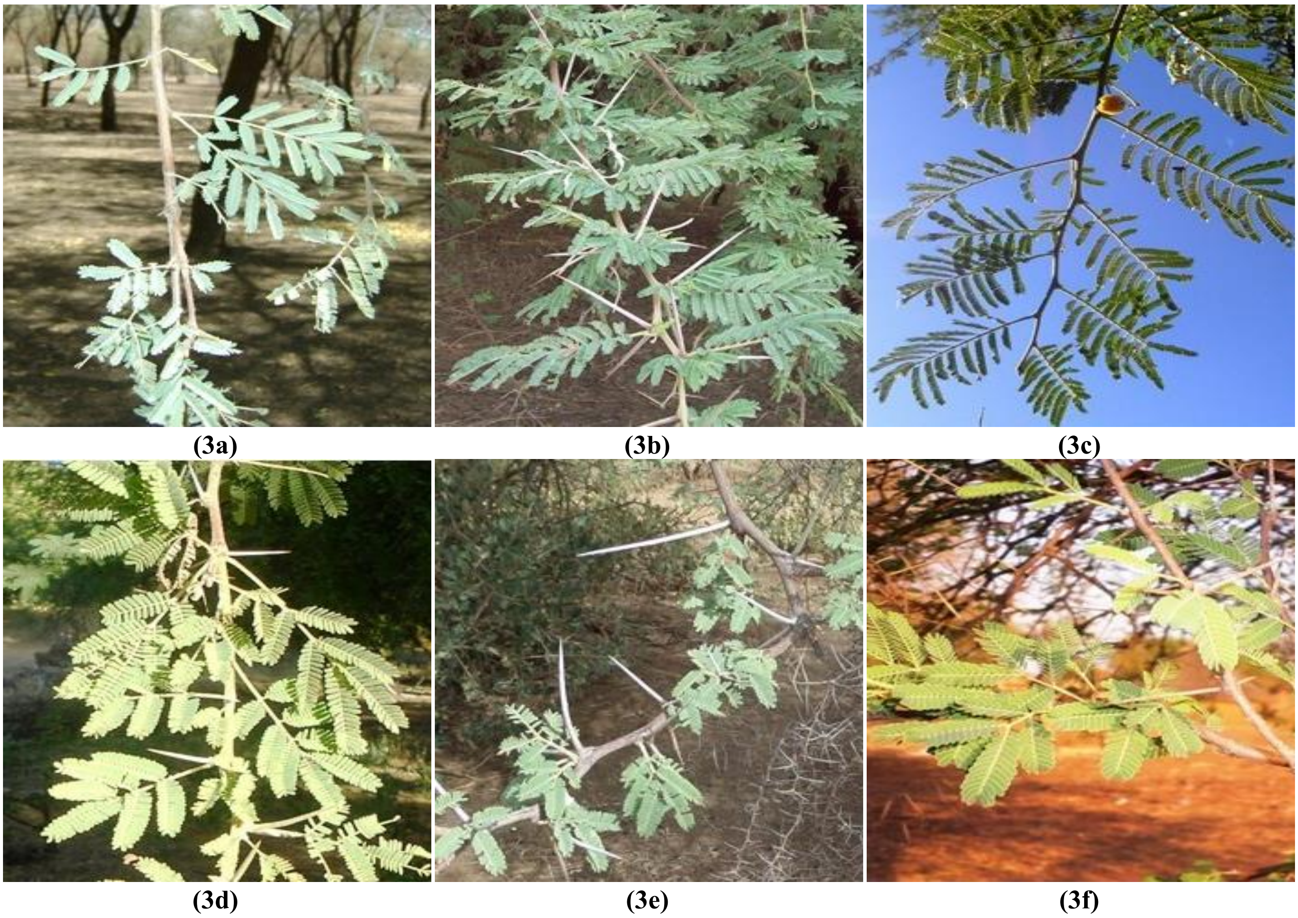

Fig. 3. Variation in leaves, branchlets, and stipular spines morphological characteristics among the subspecies *nilotica* (**3a**), *tomentosa* (**3b**), the new group (**3c**), and *adstringens* (**3d**, **3e**, and **3f**).

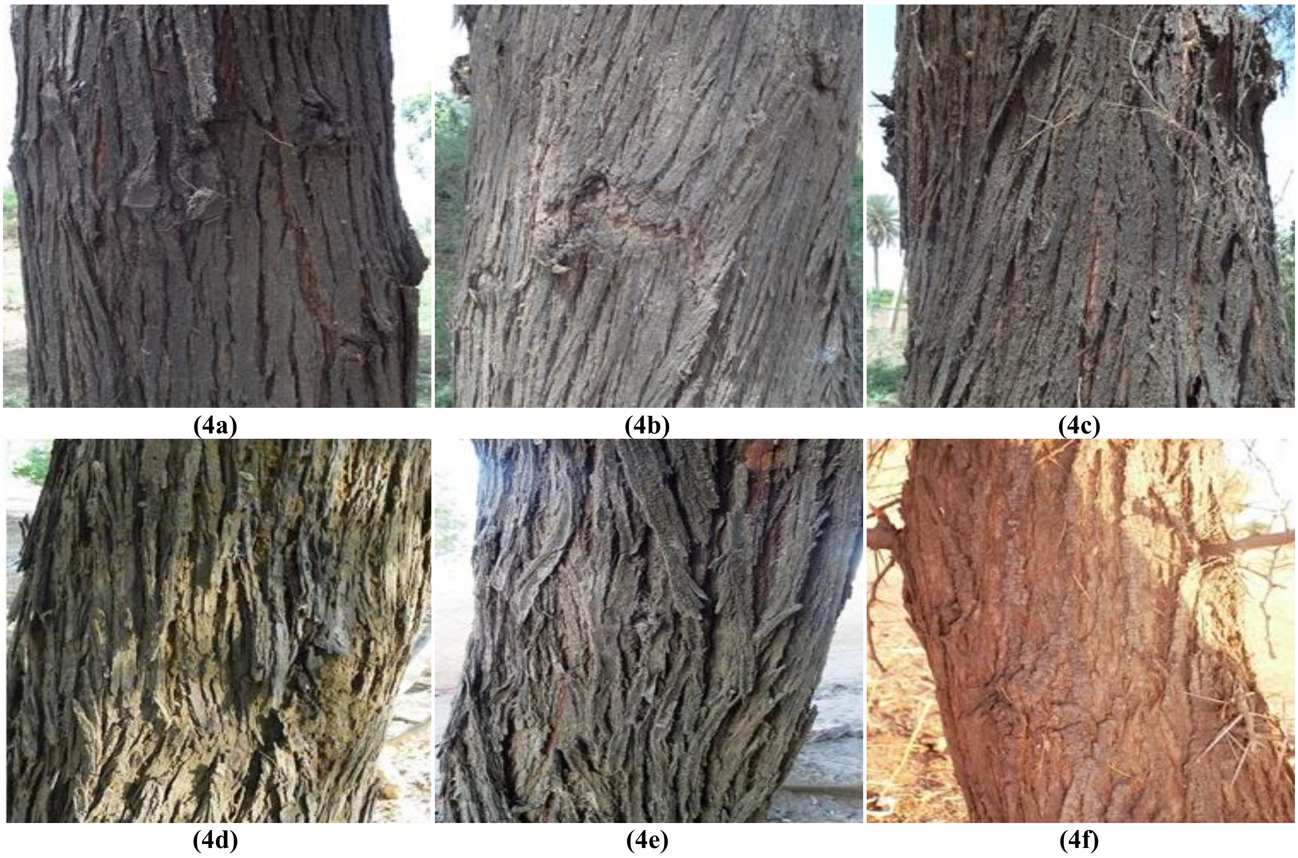

Fig. 4. Variation in characteristic of outer bark of the main stem among the subspecies *nilotica* (**4a**), *tomentosa* (**4b**), the new group (**4c**) and *adstringens* (**4d**, **4e**, and **4f**).

Table 7. Similarity index values for pod and seed quantitative traits among three subspecies of *Vachellia nilotica* and the new group in the Sudan, generated by Spearman's rank coefficient.

Genotypes	*Adstringens*	*Nilotica*	*Tomentosa*	New group
Adstringens	1.00	0.94	0.96	0.96
Nilotica	0.94	1.00	0.9909	0.99
Tomentosa	0.96	0.99	1.00	1.00
New group	0.96	0.99	1.00	1.00

Taxonomic relationship among the identified sub-taxa of *Acacia nilotica* (*Vachellia nilotica*) based on pods, seeds, and vegetative parts morphological traits: The taxonomic relationship among the three identified subspecies and the new group of *A. nilotica* was estimated by calculating the similarity among each pair of them, based on the quantitative and qualitative data of the measured vegetative traits, using Spearman's rho coefficient (Table 7).

The phenogram resulting from the Unweighted Pair-Group method with the Arithmetic mean (UPGMA) clustering of the similarity matrix of morpho-metric and qualitative traits of the pods and seeds suggested a good morphological differentiation (Table 7). However, the new group in the first sub-cluster had close similarity in pods and seeds morphology to the subspecies *tomentosa* (100%) than to the subspecies *nilotica* (99%) in the same sub-cluster. The second cluster comprised only the subspecies *adstringens* as a morphologically distinct group with an average similarity of 94% to the subspecies *nilotica* and 96% to both *tomentosa* and the new group (Fig. 5).

Also, the results showed high similarity in vegetative traits among the studied sub-taxa and the new group of *Acacia nilotica* with an average of 93%, ranging between the lowest average similarity of 46% between *adstringens* and the new group and the highest one with 99% between the subspecies *nilotica* and *tomentosa* (Table 8).

The morphometric phenogram based on vegetative data (Fig. 6) showed two main clusters among the studied sub-taxa, the first sub-cluster comprised only the subspecies *adstringens* as a distinct group, while the subspecies *nilotica*, *tomentosa,* and new group showed close similarity in vegetative characteristics and were grouped together in the second sub-cluster.

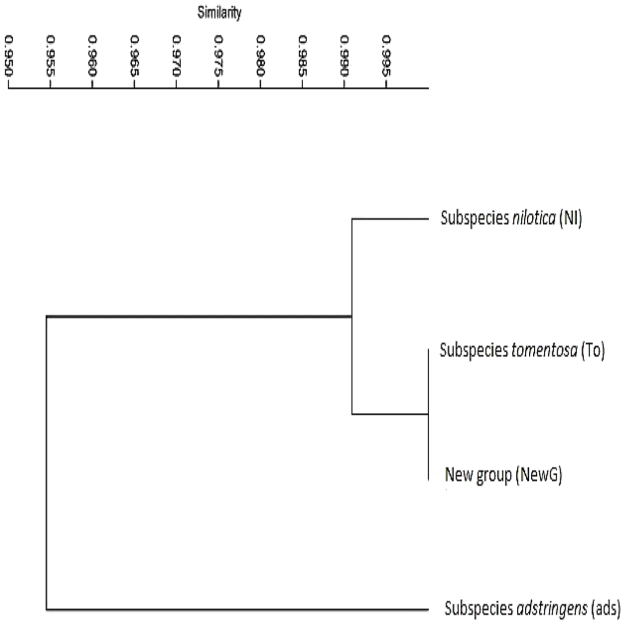

Fig. 5. Cluster phenograms based on quantitative and qualitative data of pods and seeds among the subspecies of *Acacia nilotica* and the new group.

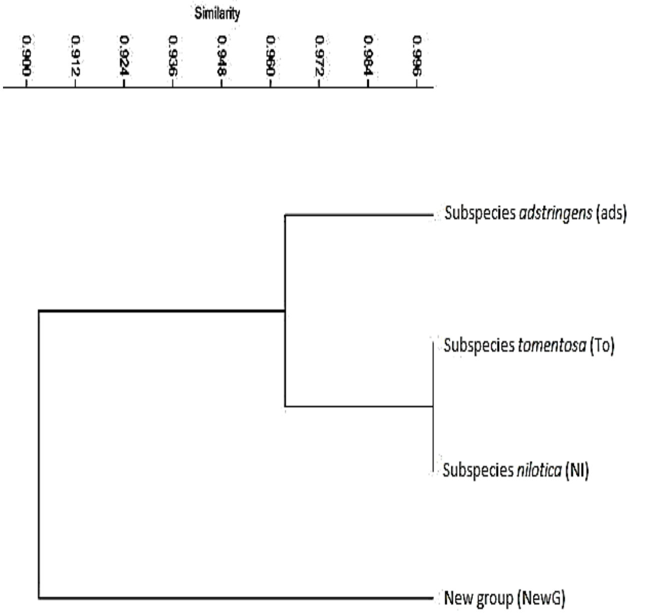

Fig. 6. Dendrogram based on quantitative and qualitative vegetative (leaves and stipular spines) traits showing the taxonomic relatedness among *Acacia nilotica* subspecies and the new group.

Table 8. Vegetative similarity index values among the subspecies of *Vachellia nilotica* and the new group generated by Spearman's rank correlation coefficient or Spearman's rho (Hollander, 1973).

Genotypes	Genotypes			
	subsp. *nilotica*	**subsp. *tomentosa***	**New group**	**subsp. *adstringens***
Subsp. *nilotica*	1	0.99	0.94	0.55
Subsp. *tomentosa*	0.99	1	0.96	0.48
New group	0.95	0.96	1	0.46
subsp. *adstringens*	0.55	0.48	0.46	1

Discussion

Sudan is a vast country in Africa with diverse climatic conditions, habitats, and plant diversity. *Vachellia nilotica* is an economic forestry species with wide distribution and sustainable management in the country. It comprises nine subspecies worldwide (Nations, 2023), of which three are native to Africa and six from Indian subcontinents (Chhillar *et al*., 2002). In this study, four groups were identified during the field surveys which covered 13 variable sites. The subspecies *nilotica*, *tomentosa*, *adstringens*, and a new morphological group (intermediate in morphology between *nilotica* & *tomentosa*) were identified. The statistical analysis indicated that most significant morphological variation in pods, seeds, and vegetative traits occurs among them. Occurrence of the subspecies *nilotica*, *tomentosa*, and *adstringens* beside subspecies *subalata* has been reported previouslyon Sudan's flora (Kordodani & Ingrouille, 1991; Elkhalifa *et al*., 2005; Darbyshire *et al*., 2015). Additionally, the newly recognized group was identified mainly in sites where both the subspecies *nilotica* and *tomentosa* are present. This new group may be due to the natural hybridization that has been reported to occur among the subspecies of *Acacia nilotica* (Ali & Qaiser, 1980). Brenan stated that the higher total diversity and population differentiation noticed in subspecies *tomentosa* could be due to the occurrence of certain hybrids in the populations (Nations, 2023). The four subspecies *tomentosa*, *nilotica*, *adstringens* and *subalata* are known to grow sympatrically over extensive areas in their natural range in Sudan and to have higher ploidy levels. It is clear from the results that shape, size, texture, and colour of the pods and seeds as well as the number of pinnae per leaf are of a higher taxonomic value because they showed high variability among the three subspecies and the new group than the vegetative traits (branches, branchlets, stem outer bark), which are overlapping. This result agrees with the findings of Brenan (Nations, 2023), Ali and Qaiser (Ali & Qaiser, 1980) who reported that the subspecies of *Acacia nilotica* can be identified using the plant habit and the shape of indumentums in both fruits and branchlets and the constriction of fruits between the seeds. Furthermore, Mahmood et al. (Mahmood *et al*., 2005) reported that the floral and fruiting traits are genetically controlled. Therefore, they are less liable to change, than certain vegetative expressions that may evolve very quickly in response to the variability of habitat and seems likely to be environmentally controlled. Thus, genetically controlled expressions may not show any differentiation despite the variability of the environment (Mahmood *et al*., 2005). *Acacia nilotica* is an available tree that exhibited different pod shapes and grows in different habitats. However, Ndoye-Ndir *et al*., (2008) grouped the subspecies based on pods variation and genetic variation at the DNA level into the necklace-like pods (*tomentosa*, *cupressiformis*, *indica*, *nilotica*, *jacquemontii*, and *subalata*) and the non-necklace-like ones (*adstringens* and *leiocarpa*). Many factors may have contributed to the divergence; for example, the reproductive and vegetative traits usually manifest different patterns of variations (Okello *et al*., 2018). The number of fruits and seeds is an important biological property, which influences the ability to generatively propagate particular tree species (Okello *et al*., 2018). Although, the new group among the other subspecies showed the longest pods 14.92 ± 7.45 cm with a high number of seeds 11.20 ± 1.54 while pods in the subspecies *adstringens* were shorter in length 11.37 ± 2.27 cm with a low number of seeds 9.27 ± 1.61, wide breadth 1.80 ± 0.14 cm and wide constriction between the seeds 1.59 ± 0.70 cm. In agreement with Okwu *et al*., (2017) and Jaspher *et al*. (2018), the longer the fruits of *Tamarindus indica*, the more the fruit pulp and the likelihood of the fruit producing many seeds, or the shorter the fruits, the less the amount of fruit pulp and the more the likelihood of the fruit producing few seeds. More and above, the average seeds weight (gm) in this study varied among the subspecies to 0.2331 ± 2.36 gm in the subspecies *adstringens* to 11.49 ± 0.83 in the subspecies *nilotica*, 11.74 ± 1.19 gm in the subspecies *tomentosa* while it was intermediate in the new group 11.79 ± 0.74 gm. In agreement with Ammar (2014), subspecies *adstringens* had significantly higher total seed weight (0.21784 gm) and seed coat (0.14632 gm) than those of *tomentosa* (0.12526 and 0.066041 gm, respectively). Also, Ammar (2014) stated that the weight of the seed coat of *adstringens* was more than double that of *tomentosa*, which resulted in the observed higher total seed weight and volume. The significance of the higher weight and volume of *adstringens* may be related to the relative dryer prevailing condition in its natural range. Studies on fruit and seed morphological traits in many tree species` natural populations have also been reported (Okwu *et al*., 2017; Abasse *et al*., 2011; El-Ayadi *et al*., 2012; Mkwezalamba *et al*., 2015). In accordance with the findings of Raddad (2007), it is certainly the case that many factors contribute to the relative amounts of variation observed among populations of the same species, particularly when populations have been separated for a long period of time.

Conclusion

The study demonstrated the diagnostic importance of mature pods, seeds, leaves, texture of bark, and branchlets as morphological features for the subclassification of *Vachellia nilotica* (Syn: *Acacia ilotica*). Three subspecies *V. nilotica* subsp. *nilotica*, *V. nilotica* subsp. *tomentosa,* and *V. nilotica* subsp. *adstringens* were identified among the population in Sudan. In addition, a new morphological group of hybrid origin in the mixed plantations of the subspecies *nilotica* and *tomentosa* was reported for the first time. It possessed intermediate characteristics of pods, seeds, leaves, and habits between *nilotica* and *tomentosa* subspecies, indicating a potential hybridization among the subspecies.

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