CHAPTER THREE

Variations in morphometric characteristics and effect of storage and temperature on neem seeds from different provenances in Sudan.

3.1 Introduction

The neem trees have been grown successfully in all parts of Sudan. Nevertheless, extensive are planted plantings on regular production basis have not been carried out yet. However, small scale trials were tried in Southern Sudan in addition to Eltuboun in Western Kordofan and Eldebabat in Northern Kordofan but no concrete results about its success or failure as a plantation tree has been published (Mahjoub, 2001). Various ecotypes of *Azadirachta indica* exhibit variation in several characters (Arora, 1993).

Seed storage is very important to secure good quality seeds for planting programs whenever needed. Seed longevity depend on genetic and physiological factors as well as storage conditions (Coronado, *et al.*, 2007).The most important factors that influence storage are temperature, moisture, seed characteristics, micro-organism geographical location and storage structure (Govender, *et al.*, 2009). It is necessary to improve methods that increase potential seed longevity in storage (Coronado, *et al.*, 2007).

Seed viability can be extended by cold or dry storage at seed moisture content below 5% (Huang *et al*, 2003). Dormancy plays a major role in regulating germination in dry forest species.

Measurements of the morphometric characteristics are usually carried out on samples randomly drawn from each seed source. Seed length and width were measured using suitable devices, while the number of seed per kilogram, moisture content and germination was determined according to standard seed testing assertion roles (ISTA) procedures.

Seed weight is essential in calculating requirements for a given planting project. It is indicated either as number of seed per kilogram or as weight in grams of 1000 seeds. Seeds weight is a variable character within species and it is influenced by genetic, developmental and environmental factors (Schmidt, 2000).

Seed size is known to affect various aspects of plant life (Milberg and Lamont, 1997). It influences the dispersal, seed water relations, emergence, establishment, survival and growth of seedlings (Wunderle, 1997).

Neem seeds are known for their short viability (2-3 months) and poor storability. It is classified as recalcitrant seed (with high moisture content). (Wunderle, 1997).

Therefore the objective of this research was to identify variation in storability of neem seed among neem provenances that may be affected by environmental or genotypic factors.

3.2 Materials and methods

3.2.1Seed sources

Mature and healthy yellow fruits of neem *Azadirachta indica* were collected from seven different seed sources in various parts of Sudan. The seed sources used were namely provenances of Elfasher, Elobied, Bara, Eldalang, Abassya, Senga and Gedaref representing western, central and eastern parts of the country. Table 3.1 and Figure 3.1.

`Table 3.1 Details of the area of provenances used in the study.

Provenances	Latitude	Longitude	Altitude (m)	Rainfal l (mm)	Temperature (°C)	
					Max	Min
Elfasher	13° 37′ 50″ N	25° 21′ E	700	186.7	34.1	16.5
Elobied	13° 11′ N	30° 13′ E	587	318	34.6	20.6
Bara	13° 40′ 56″ N	30° 21′ 55″E	613	110.5	34.6	20.6
Eldalang	12° 05′ N	29° 65′ E	688	625	35	18.5
Abassya	120° 12′ N	31° 17′ E	376	625	35	18.5
Senga	13° 9′ 7″ N	33° 55′ 35″ E	439	512	36	20
Gedaref	14° 2′ N	35° 23′ E	580	750	47	17

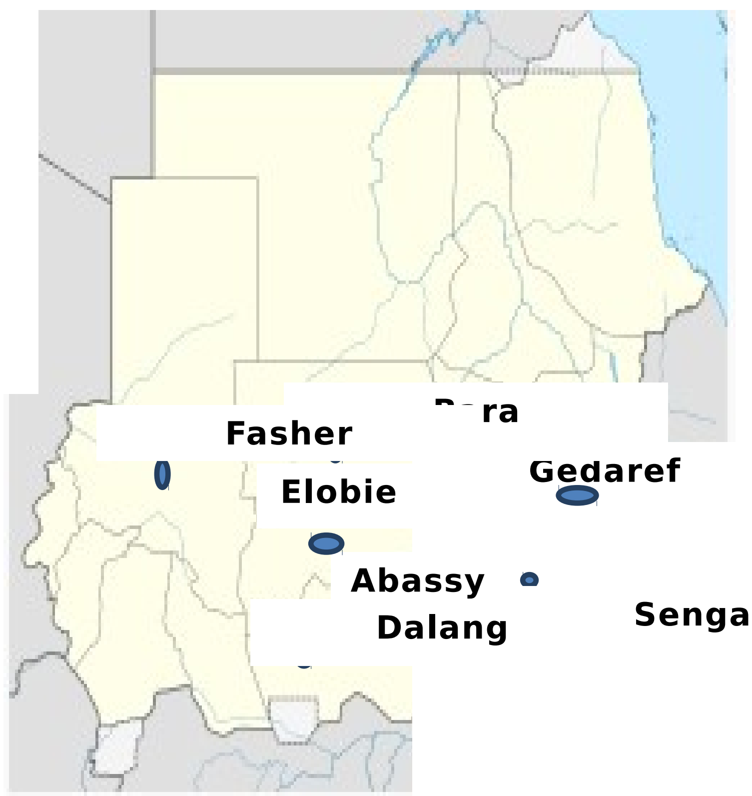


Figure 3.1 Locations of the seven pro
study

(Wikipedia, 2013)

3.2.2 Fruit collection and processing

Mature and healthy neem fruits were collected from selected trees grown in plantations at the specified geographic areas (Table 3.1). Four sites within each area were selected randomly and a sample of six trees was marked in each site for fruit collection. Fruits were collected from the tree crown by shaking with long hooked sticks and by hand from under the trees. In total one kilogram of fruits were collected from the marked trees. Collected fruits were then transported to the National Tree Seed Centre at Elobied-Sudan, where they were cleaned in the processing room and dried under shade in dry weather. Seed processing was carried out in accordance with the International Neem Network recommendations (Thomsen and Souvannavong, 1994). The fleshy neem fruits were gently squeezed after collection and seeds were extracted while in water. This was repeated until all the pulp is separated in water then seeds were allowed to settle and the pulp was poured off. After washing, seeds were dried in thin layers on absorbent sheets with circulating air in the shade.

3.2.3 Seed storage

The experiment was conducted at the Regional Tree Seed Centre-Elobied, North Kordofan.

- Cool storage: seed were stored at 12$\pm$ 1 ^{0}C in airtight containers for the whole period at the storage.

- Normal store seed were stored at normal room temperatures 25 – 30 C ^{o} in cotton sack under good aeration.

Seed viability and moisture were monitored and recorded at four month intervals starting from initial viability and moisture at collection.

Under laboratory conditions, samples of 15 seeds each were drawn from the seed lots of each of the seven provenances. Seeds were then sown in germination trays filled with sand and they were replicated four times and watered daily in a germination room with temperature; 28-32 C^0 and 12 hours light from florescent lamps. Germination percentage was recorded every week for four consecutive weeks and the final cumulative germinated seeds were divided by total number of sown seeds multiplied by one hundred. For percentage germination in total three germination tests were performed during three months duration after dividing into two seed lots of each provenance and each complete divided seed lot was packed. It was then either stored at a normal room temperature (25-30°C) or in a cold store at (12± 1 °C) in air light containers. Seed samples were then drawn from the stored seed every month and were subjected to a germination test.

Germination % was recorded every week for four weeks and the final germination % was calculated as follows:

$$\text{Germination (\%)} = \frac{\text{Total germination in all replicates}}{\text{Total no. of sown seeds}} \times 100$$

3.2.4 Determination of non-morphometric seed characteristics

3.2.4.1 Number of seed per kilogram

To determine this characteristic in the laboratory, three hundred sound seeds were taken at random from the seed lot and divided into 3 replicates. Seed weight per 100 seeds was recorded using a sensitive balance.

The number of seeds per kilogram was calculated using the formula:

$$\text{Number of seed per kg} = \frac{\text{Number of seeds x 1000}}{\text{Average seeds weight per gram}}$$

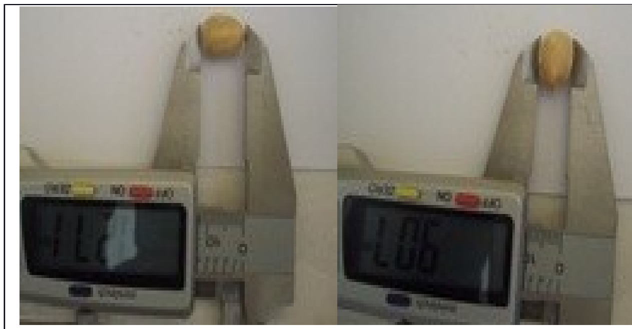
3.2.4.2 Seed moisture content

Moisture content was measured by using oven drying methods as prescribed by ISTA. Two empty containers were weighed. Samples of seeds were drawn for moisture content, and seed were cut into small pieces before drying to assure that moisture can escape from the interior. Ten gram of seeds were weighed and put into containers, the weight of the container is not included. Seeds were placed in an oven at 83 °C for 24hours. After drying, containers were placed in desiccators while cooling. Samples were allowed to cool in an incubator for 45 minutes and then weighed. The moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

3.2.5Morphometric characteristics of seeds

Seed length and width were measured in millimeters using a digital caliper. Thirty seeds of each provenance were used and were replicated four times then the averages were calculated.



Length(mm) width (mm)

Seeds msured by digital caliper

Analysis of variance was carried out using SAS statistical software version 6.12 (SAS Institute Inc., 1996) to determine the significance of variations among the provenances. Duncan Multiple Range Test was used to separate the means.

3.3 Results

Morphomatric characters:

Analysis of variance of seed morphometric data showed highly significant differences between the provenances on seed length and width. However, there were no significant differences between the provenances in seed weight as shown in Table 3.2.

3.3. 1 Seed length

Seed length ranged from 14.16 to 11.10 mm and these were recorded by Abasaya and Elfasher provenances respectively (Table 3.3). Elfasher provenance had significantly longer seed compared with the other six of the provenances. In this connection Elobeid provenance ranked in the second place recording seed length of (13.36 mm). However the provenances of Senga and Eldalang had no significant differences between them because they recorded recording lengths of (12.32mm) and (12.22mm) respectively and ranking in the third place. On the other hand Bara provenance recorded significantly shorter seed length (11.68 mm) as compared to both Senga and Eldalang provenances but at the same time it was significantly longer than both Gedaref (11.43) and Abassya provenances (11.10). However, Gedaref provenance recorded significantly longer seed compared to Abasaya which in ranking at the bottom of the list.

3.3.2 Seed width

The same trend with the seed length seems to be the case with the seed width characteristic. In this connection Elfasher provenance recorded a significantly

Table 3.2 ANOVA on Seed Size Characteristic of *Azadirachta indica* provenances in Sudan

Source of variation		Width			Length			Weight			Number of seed (kg)		
Provenance	df	SS	M S	F Value	SS	MS	F Value	SS	MS	F Value	SS	M S	F Value
	6	452.94	75.49	53.45^{***}	81.30	13.55	51.46^{***}	594.58	99.10	0.85^{n}	62530438	10421739.7	188.85^{***}

Table 3.3 Variation in mean seed characteristics of seven *Azadirachta indica* provenances in Sudan

Ranks	Number of seed (per kg)	Ranks	Seed width (mm)	Ranks	Seed length (mm)	Provenance
2	6301[b]	5	5.87[d]	3	12.22[c]	Eldalang
4	4976[d]	3	6.13[c]	7	11.10[cd]	Abassya
5	4665[e]	2	6.70[b]	3	12.32[c]	Senga
1	6696[a]	4	5.93[d]	5	11.43[e]	Gedaref
7	3438[g]	1	6.95[a]	1	14.16[a]	Elfasher
3	5721[c]	2	6.57[b]	4	11.68[de]	Bara
6	4386[f]	3	6.18[c]	2	13.36[b]	Elobied

Means with the same letter on the same column are not significantly different at p= 0.05 by Duncan New Multiple Range Test.

Longer seed width (6.95 mm) and ranked first compared to the rest of the provenances. However, Senga and Bara provenances showed no significant differences and ranked second place recording seed widths of (6.70 mm) and (6.57 mm), respectively. However, Eldalang and Gedaref provenances showed no significant differences between them as well as they were recording the smallest seed widths (5.87mm) and (5.93mm) compared to the rest of the provenances as shown in Table 3.3.

3.3.3 Number of seeds per kilogram

The seven provenances showed significant differences between them in this character. The number of seeds per kilogram ranged from (3438 to 6696) seeds. The biggest number of seed 6696 was obtained from Gedaref provenance ranking at the top, while the least number of seeds 3438 was recorded by Elfasher provenance. Eldalang provenance ranked in the second place with 6301 seeds per kilogram and Bara provenance ranked third recording 5721 seeds per kilogram. The remainder of the provenances were however, ranked intermediate, but no two provenances showed similarity with each other in this characteristic as shown in Table 3.3.

3.3.4. Seeds storage

3.3.4.1 Moisture contents and germination under normal storage

The seven provenances showed significant differences between them in these characters. Abassya provenance recorded significantly higher seed moisture content (9.7%) compared to the rest of the provenances. However, Elfasher, Elobied and Senga provenances showed no significant differences between then. They ranked in the second place recording (8.7%), (8.5%) and (8.2%) respectively. However, Gedaref and Eldalang provenances showed no significant differences between them, recording significantly lower moisture content (7.5%) and (7.4%) compared to the rest of the provenances as shown in Table 3.4 and Figure 3.2.

The germination % varied significantly among the provenances, Bara, Eldalang and Elobied provenances showed no significant differences between then. They ranked in the first place recording (70.6%), (65.5%) and (60.3%) respectively. Elfasher, Abassya and Senga provenances ranked in the second place recording (55.2%), (50.9%) and (48.8%) respectively. However, Gedaref provenance recorded lower germination (42.3%) as shown in Table 3.5 and Figure 3.3.

3.3.4.2 Moisture contents and germination under cool storage

The seven provenances showed significant differences in this which Abassya and Elfasher provenances recorded significantly higher moisture content (9.9%) and (9.8%), respectively. However, Elobied provenance recorded (9.3%) ranking in the second place. Senga provenance was intermediate (8%). However, Gedaref, Eldalang and Bara provenances showed no significant differences recording the lowest moisture content (7.9%), (7.5%) and (7.4%) respectively as shown in Table 3.5 and Figure 3.2.

Germination of seed varied significantly among the provenances, Bara and Senga provenances showed no significant differences They ranked in the first place recording (72.4%) and (69.8%) respectively. Eldalang and Elobied provenances ranked in the second place recording (58%) and (54%) respectively. However, Abassya and Gedaref provenances recorded significantly lower germination (39%) and (38.6%) as shown in Table 3.5 and Figure 3.4.

Table 3.4 ANOVA on moisture content and germination % of provenances

Source of variation	df	Moisture content %			Germination %		
		SS	Mean Square	F Value	SS	Mean Square	F Value
Provenances	6	43.1	7.2	5.8***	28192.7	4698.8	15.9***

Table 3.5 Variation in moisture content and germination % of provenances

Provenances	Moisture content %		Germination %	
	Storage		Storage	
	Normal	Cool	Normal	Cool

Eldalang	7.4^{c}	7.5^{c}	65.5^{a}	58^{b}
Abassya	9.7^{a}	9.9^{a}	50.9^{b}	39^{c}
Gedaref	7.5^{c}	7.9^{c}	42.3^{bc}	38.6^{c}
Bara	7.8^{bc}	7.4^{c}	70.6^{a}	72.4^{a}
Senga	8.2^{b}	8^{bc}	48.8^{b}	69.8^{a}
Elfasher	8.4^{b}	9.8^{a}	55.2^{b}	48.9^{bc}
Elobied	8.3^{b}	9.3^{ab}	60.3^{a}	54^{b}

3.4 Discussion:

Variation in seed weight, length and width between provenances were due to evolutionary responses of seed to their specific habitats. Production of a large number of seeds is to maximize the potential fitness by producing a larger number of seeds and increase the chance of establishment of resulting seedlings through great allocation of maternal resources to individual seeds (Zhang, 1998).

Observed phenotypic variation is generally assumed to reflect the inherent genotypic variation among the provenances grown under uniform conditions. Seed characteristics delineated significant differences among and within provenances from different regions and might reflect the true genetic variations among these provenances as a response of differences in environmental variation.

The significant variation in seed morphometric characters among and within the provenances of *Azadirachta indica* may reflect the overriding impact of both environmental and genetic variation and this can be assumed to reflect true genetic variation and adaptation to different environmental conditions and soil type.

The seven neem provenances used in the present work showed variation in seed weight, length and width. The same finding was found by Abdel Khair, *et al.,* (2003) who reported that *Acacia karoo* displayed significant differences among geographical sources in seed characteristics like seeds weight, number/kg, length and width. Phenotypic variation is determined by genotype and environment interaction and is assumed to express genotypic variation when environmental conditions are controlled (Danasuk *et al.,* 1997, Westoby *et al.,* 2002 and Raddad, 2007).

It has been shown that seed width was also affected by other factors. As reported by (Fenner, 1985), variability in seed size (width, length and thickness) was probably a consequence of a compromise between the requirements for dispersal (which would favor small seeds) and their requirements for seedling establishment (which would favor larger seeds) (Debeaujon *et al.,* 2000; Yanlong *et al.,* 2007; Souza and Fagundes, 2014).

Seed width is usually one of the parameters that remain not variable within or among seed lots from deferent provenances, while seed length is mostly variable characteristics affected by environmental condition (Mahjoub, 2001).

Neem is a species of broad phenotypic plasticity expressed in the wide spread of the tree in the different climate zones and habitats, therefore it can be concluded that significant differences among provenances of neem in seeds characters are direct responses of differences in their natural habitats. This is shown in the deciduous habit acquired in the dry tropics while it is classified as an evergreen tree in India and other Asian countries (Anonymous, 1986; Girish and Bhat, 2008 and Orwa, *et al.,* 2009).

The germination and moisture content of seeds showed variation on neem provenances storage under normal and cool conditions for three months. Neem seeds cannot be stored successfully for long periods as they are recalcitrant Seeds. Neem seeds are reputed as sensitive to low temperature and chilling damage and death may occur if stored in low temperature (Bisht and Ahlawat, 1999).

Neem seeds are reputed to have limited desiccation tolerance and relatively short storage longevity (Gamene *at el*., 1996; Hong and Ellis, 1998; Sacande *at el*., 1996).

The critical water content below which the seeds will not germinate is difficult to determine, because such dry seeds are extremely sensitive to imbibitional stress. This stress can at least partly be alleviated by imbibitions at elevated temperature (25-35 ^{0}C) (Sacande *et al*., 1998).

Other studies undertaken by Gaméné *et al*. (1996) and Sacandé *et al*. (1996) showed that neem seeds are intermediate in seed storage behavior when compared with orthodox and recalcitrant seeds. These clear differences in the ability of seeds of this species to retain viability might be due to factors such as provenance, storage conditions and seed developmental stage. When they originate from tropical climates, such intermediate seeds are often chilling sensitive (Sacande *et al*., 1998).