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Variation in Morphometric Characteristics and Effect of Storage and Temperature on NeemSeeds from Different Provenances in Sudan

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ABSTRACT

The main aim of the present work was to assess the variation of the neem provenances and their extended areas and the adaptation to the different eco-climatic conditions in its occurrences in Sudan. The specific objective was to study the variation in seed morphometric characteristics among seven neem (*Azadirachtaindica* A. Juss) provenances western, central and eastern parts of the country. Investigated the seed morphometric and physiological characters. The obtained data from the three experiments was analyzed using analysis of variance and SAS software version 6.12 and the means were separated using Duncan New Multiple Range Test. The analysis showed significant variations between the provenances in most of the measured variables indicating that neem tree in Sudan is adapted to the different eco-climatic zones in Sudan. In this regard, Elfasher provenance is excelled in having significantly superior morphometric characteristics (seed length, seed width) and recording the least number of seed per kilogram, which is vice versa in Gedaref provenance. The storage and moisture content experiments showed that Abassya provenance seeds have the highest moisture content (9.7%) with least germination percentage (39%) in normal conditions. While seeds from Bara, Senga recorded the highest germination percentage (72.4%, 69.8%). In this study it was clear found that the neem tree in Sudan, although it is generated from one tree (in Shambat), it established in different provenances in various geographical locations and ecotype over Sudan, due to its adaptation ability.

Keywords: *Azadirachtaindica*, morphometric, storage, provenance variation, adaptation**.**

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 yet been published (Mahjoub, 2001).

Various ecotypes of *Azadirachtaindica* exhibit variation in several characters (Tomarand Kaushik, 2011).Seed storage are 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 the International Seed Testining Association (ISTA) standard seed testing procedures (ISTA, 1993). 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 morphometric characteristics and effect of storage conditions on neem seeds from seven provenances in Sudan.

MATERIALS AND METHODS

Seed Sources

Mature and healthy yellow fruits of neem are collected from seven different seed sources in various parts of Sudan. The seed sources used were namely: Elfasher, Elobied, Bara, Eldalang, Abassya, Senga and Gedaref representing western, central and eastern parts of the country (Table 1) Seeds from the different sources were collected during the season 2011 from the period March to April.

Table1: Details of the area of provenances used in the study

Provenances	Latitude	Longitude	Altitude (m)	Rainfall (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

Fruit Collection and Processing

Mature and healthy neem fruits were collected from selected trees grown as avenues and shade trees at the specified geographic areas (Table.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 using 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, North Kordofan State, 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 (INNW) (Thomsen and Souvannavong, 1994). The fleshy fruits were gently squeezed after collection and seeds were extracted while in water. This was repeated until all the pulp is separated then seeds were allowed to settle and the pulp was poured off. After washing, seeds were spread in thin layers on absorbent sheets with circulating air in the shade and left to dry for four days.

Seed Storage

The experiment was conducted at the Regional Tree Seed Centre, Elobied, NorthKordofan. Two storage regimes were used:

- Cool storage: seed were stored at 12±1˚C in airtight containers for the whole period at the storage (how many days).
- Normal store: seed were stored at normal room temperatures 25 – 30◦C 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 which was carried out before seed storage.

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 at temperature of 28-32◦C and12 hours light from florescent lamps. Germination percentage (G%) was recorded every week for four consecutive weeks. The final cumulative germinated seeds were divided by total number of sown seeds and multiplied by one hundred to obtain G % .In total, three germination tests were performed during three months duration. Afterwards, the seeds lots of each seed source was divided into two seed lots and packed. One of these seed lot was stored at a normal room temperature (25-30◦C) and the other was stored in a cold store at (12± 1◦C) in air light containers. Afterwards seed samples were then drawn from each of the stored seedlots every month to determine germination %.Seed germination % was recorded every week for four weeks and the final germination % was calculated as follows:

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

Determination of non-morphometric seed characteristics

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:

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

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 weighted and put into containers, the weight of the container is not included. Seeds were placed in an oven at 83˚C for 24 hours. After drying, containers were placed in desiccators while cooling. Samples were allowed to cool in an incubator for 45 minutes and then weighed. Seeds moisture content was calculated as follows:

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

Morphometric 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 (Fig. 1).

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.

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 2.

Fig. 1: Seeds msured by digital caliper Length (mm), width (mm)

Seed Length

Seed length ranged from 14.16 to 11.10 mm and these were recorded by Abasaya and Elfasher provenances, respectively (Table 3). Elfasher provenance had significantly longer seed compared with the other six provenances. In this connection Elobeid provenance ranked in the second place recording seed length of (13.36 mm). However, 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.

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 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.

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.

Seeds Storage

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 4.

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 4.

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 Table4.

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 4.

Table 2: ANOVA on Seed Size Characteristic of *Azadirachtaindica* provenances in Sudan

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

Table 3: Variation in mean seed characteristics of seven *Azadirachtaindica* provenances in Sudan

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

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

Table 4: 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}

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 *Azadirachtaindica* 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; Girishand 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 *et al.,* 1996; Hong and Ellis, 1998; Sacande *et al.,* 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°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).

CONCLUSION

Highly significant variation in seeds morphometric characteristics among provenances of *Azadirachtaindica*, may reflect both environmental and variation and adaptation to different environmental conditions and soil types. Neem is a species of broad phenotypic plasticity expressed in the wide spread occurrence 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. Cool and normal storage conditions decreased seed germinability by increased storage time. Neem seed can be classified as recalcitrant and cannot be stored successfully for long periods. The seed of neem seems to be sensitive to low temperature, chilling damage and death may occur if stored in low temperatures.

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