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Variations in Nursery Seedlings Growth Performance of *Azadirachta Indica* 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 seedlings growth performance among seven neem (*Azadirachta indica* A. Juss) provenances western, central and eastern parts of the country. The experiment was performed to fulfill the objectives of the study. The experiment was conducted at the lab of the nursery of the Regional Tree Seed Center, Elobied Sudan. The experiment investigated was conducted in seedling growth in nursery pots using Completely Randomized Design (CRD) with five replicates for seedling parameters measurement (root collar diameter, shoot length, root length, root fresh weight, number of leaves, leaf area, shoot/root length ratio, root dry weight, root fresh weight). The obtained data from the experiment was analyzed using analysis of variance and SAS software version 6.12 and the means were separated using Duncan New Multiple Range Test. The provenances showed high significant ($p<0.0001$) in growth performance (root collar diameter), however, there is no significant in leaf area among the provenances. Elfasher provenance showed significant ($p<0.0001$) in the seedling parameters (root length and leaf area), while Senga provenance is significant only with seedlings length. It is concluded that a multi site field provenance trial is required for more useful information about the studied seed sources for the eco-climatological adaptations. 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: *Azadirachta indica*, seedlings, growth performance, provenance variation, adaptation**.**

INTRODUCTION

Neem has become naturalized species in various parts of the Sudan in quite diverse ecological, climatic and edaphic conditions. The tree has been introduced to Sudan for several decades from its natural habitat in the Indian sub-content. Its use was mainly as a

shade, ornamental avenue tree, despite it is well recognized as the main ingredients in different industrial and pharmaceutical uses in India (Wang *et al*., 1993; Abutaba *et al.*, 2014). Neem is such a versatile resource that can be used in different rural areas in the Sudan. Creating plantation and as a consequence related industries based on neem as a basic raw material or ingredient can be established in these areas thus the tree can contribute to rural development and open opportunities for more jobs and economic development (Wang *et al.*, 1993; Schmutterer, 1995; Kumar and Mishra, 2007). It has been observed that neem growth performance showed clear differences ranging from satisfactory growth rates in some areas while it virtually showed a complete failure in other parts of the Sudan (Knothe, 2005). Reasons for such variability may be attributed to either adaptability to prevailing environmental conditions or the genetic diversity residing in the germ plasm of the different ecotypes and provenances evolved during the sequence of its naturalization since its introduction about one century ago. To fully utilize the rich resource of neem in the Sudan a comprehensive gynecological study supported by experimental work and sufficient trials to support or reject the hypothesis of the existence of or nonexistence of different provenances and genotypes is imperative. Such work will aid in decision making concerning existence of real differences in growth attributes that can be related to provenances variation or to adaptation of the same genotypes to the various ecosystems where it grows (Knothe, 2005). Appropriate neem plantations can thus be established in the right place to insure good growth rates, satisfactory production within the thought rotation and accordingly full industrial, pharmatical and other uses can be planned concurrently. It is well established that many tree species with a range of geographical diversity exhibit physiological, morphological, biochemical and genetic variations as an adaptation to varying environmental conditions (Knothe, 2005). Therefore the objective of this study was to examine the seedlings growth performance of *Azadirachta indica* provenances collected from seven different geographical locations in Sudan.

MATERIALS AND METHODS

The study was conducted in the Regional Tree Seed Center nursery at Elobied, using seeds from the seven provenances as indicated in (Table 1). Seeds were directly sown in polythene bags (30 × 20 cm size) with 2-3 seed sown per bag. The bags were filled with a mixture of clay and sand (2:1) as planting medium. A completely randomized design (CRD) with five replications was utilized.

Table 1: Details of the areas 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

Data Collection

The seedlings were grown for a period of one month under 50% shade and were daily watered, and given care and protection in the nursery. Measurements of the growth attributes were then performed after 30 days. Root collar diameter (mm) was measured using an electronic caliper. Seedling height was measured as the distance between the root collar and the tip of the terminal leader, number of leaves per seedling was counted and leaf area (cm^2) was measured using a systematic leaf area meter. Samples of three seedlings were randomly selected from each provenance. From each sample three leaves were randomly taken from the upper, middle and lower part of the seedling for leaf area measurement as described by (Chen *et al.*, 2002). Destructive sampling was applied to measure the root length, root weight, shoot length and shoot weight. After removal of the rooting media by washing roots under running water seedlings were cut at root collar level beneath the cotyledon scars and their entire length was measured up to shoot tip as seedling height in centimeters. The root length

was measured at the point of cotyledon scars and lowest end of roots. Weighing of fresh shoot and root was carried out using the displacement method as described by Mitchell, (1968). Oven dry weight was determined by drying at 80±1°C for 48 hours. Shoot: root raio was then calculated on dry weight basis.

Data Analysis

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

RESULTS

Analysis of variance showed very high significant ($p< 0.0001$) differences among the provenances for shoot/root length ratio, root collar diameter, root dry weight and root length. The provenances also showed highly significant ($p<0.001$) differences in shoot/root fresh weight ratio and shoot length. The analysis revealed only significant ($p < 0.05$) differences between the provenances in shoot dry weight and root fresh weight variables (Table 2). There were no significant differences among provenances in leaf area shoot fresh weight and shoot/root dry weight ratio.

Shoot Length

The shoot lengths of the seedlings varied significantly among the provenances. Senga provenance recorded the longest seedling shoot length (38.80 cm) followed by Elobied provenance which attained a length of (35.20 cm). Seedling shoot length ranged from (24.90 cm) to (38.80 cm) recorded by Gedaref and Senga provenances, respectively. Abassya provenance ranked in the third place recording a mean shoot height of (30.80 cm). The provenances of Elfasher, Eldalang and Bara ranked in the fourth place with no significant differences between them recording shoot length of (27.90, 27.10 and 26.05 cm) respectively. Gedaref provenance had the lowest ranking between the provenances in recording only (24.90 cm) as depicted in Table 3.

Root Collar Diameters

The root collar diameter ranged from (2.70 to 3.78 mm) and was recorded by Gedaref and Elfasher provenances respectively. However, Elfasher provenance had significantly bigger root collar diameter compared to all provenances. Abassya ranked in the second place and was significantly bigger than the remainder of the provenances. It recorded (3.36 mm). On the other hand Elobied provenance was significantly bigger than the remainder of the provenances recording (3.09 mm) and ranking fourth. As shown in Table (2) all the rest of the provenances showed significant differences between them and no similarities were found between any two provenances. Eldalang provenance ranked in the second lowest ranking place with a root collar diameter of (2.80 mm) while Senga and Bara were intermediate recording (2.93mm) and (2.85 mm) respectively as shown in Table 3.

Root Lengths

The provenances showed significant variations in root length characteristic. Elfasher provenance had significantly longer root (40.70 cm) compared to the remainder of the provenances, however Eldalang provenance ranked second and was also significantly different from the other five provenances recording a length of (35.8 cm). Elobied and Gedaref provenances showed no significant differences between them, recording (31.30 cm) and (30.60 cm) respectively but however, they were significantly longer than Abassya, Bara and Senga provenances. On the other hand the provenances of Abassya, Bara and Senga had no significant differences between them in root length characteristic. They recorded (29.60), (28.80) and (28.20 cm) respectively as shown in Table 3.

Shoot / Root Length Ratio

Elfasher provenance recorded (1.5) significantly highest shoot/root length ratio of the provenances. Eldalang provenance was not significantly different from Elfasher and Gedaref provenance ranked in the second place and recorded (1.4). However, Gedaref provenance was not significantly different from Elobied provenance (1.2). Senga provenance came in the lowest ranking place with a shoot/root length of (0.7) as shown in Table 3.

Root Fresh Weight

Elfasher provenance recorded (5.03) significantly bigger root fresh weight of the provenances. However, Eldalang provenance

ranked in the second place and was significantly different recording (3.19). On the other hand the provenances of Senga, Elobied, Abassya and Bara and had no significant differences between them in root fresh weight characteristic. They recorded (2.92), (2.61), (2.44) and (2.02) respectively. Gedaref provenance came in the lowest ranking place with a root fresh weight of (1.56) as shown in Table 3.

Table 2: ANOVA on seedlings growth characteristics (neem) *Azadirachta indica* provenances from different location in Sudan

Source of variation		Root collar diameter			Shoot length			Root length			Shoot/root length		
	df	SS	MS	F value	SS	MS	F value	SS	MS	F value	SS	MS	F value
provenance	6	104.34	17.39	75.20***	3700.08	616.68	41.53***	486.36	81.06	20.98***	3.96	0.66	13.21***
		Shoot fresh weight			Root fresh weight			Shoot/root fresh weight			Shoot dry weight		
		SS	MS	F value	SS	MS	F value	SS	MS	F value	SS	MS	F value
		250.72	69.63	9.73^{n}	23.83	3.97	2.28*	105.21	17.54	4.27**	4.62	0.77	3.10*
		Root dry weight			Shoot/root dry weight			Leave number			Leaf area		
		SS	MS	F value	SS	MS	F value	SS	MS	F value	SS	MS	F value
		8.81	1.47	7.80***	63.40	10.57	1.24^{n}	486.36	81.06	20.98^{n}	2553.4	425.6	2.3^{n}

Table 3: Variations in seedlings growth characteristics of *Azadirachta indica* provenances from different locations in Sudan

provenance	Root collar (mm)	Shoot length (cm)	Root length (cm)	Shoot / root length	Shoot fresh weight	Root fresh weight	Shoot/ root fresh	Shoot dry weight	Root dry weight	Shoot/ root dry weight	Leaves number	Leaf area (mm^2)
Eldalang	2.80^{ef}	27.10^{cd}	35.8^{ab}	1.4^{ab}	3.3^{a}	3.19^{b}	1^{b}	1.17^{ab}	0.89^{b}	0.98^{a}	8.80^{a}	29^{a}
Abassya	3.36^{b}	30.80^{bc}	29.60^{c}	1^{d}	3.7^{a}	2.44^{bc}	0.7^{d}	1.23^{a}	0.74^{b}	1.73^{a}	10.37^{a}	29.2^{a}
Senga	2.93^{d}	38.80^{a}	28.2^{c}	0.7^{e}	3.3^{a}	2.92^{bc}	0.9^{bc}	1.25^{a}	0.80^{b}	3.80^{a}	8.17^{a}	30^{a}
Gedaref	2.70^{f}	24.90^{d}	30.60^{bc}	1.2^{bc}	2.3^{a}	1.56^{c}	0.6^{d}	0.75^{bc}	0.71^{b}	1.45^{a}	8.12^{a}	29^{a}
Elfasher	3.78^{a}	27.90^{cd}	40.70^{a}	1.5^{a}	3^{a}	5.03^{a}	0.8^{bcd}	1.26^{a}	1.77^{a}	0.73^{a}	9.41^{a}	30.3^{a}
Bara	2.85^{de}	26.50^{cd}	28.80^{c}	0.9^{de}	4^{a}	2.02^{bc}	0.7^{d}	0.67^{c}	0.69^{b}	1.03^{a}	8.23^{a}	29.5^{a}
Elobied	3.09^{c}	35.20^{ab}	31.30^{bc}	1.1^{cd}	2.3^{a}	2.61^{bc}	1.7^{a}	1.39^{a}	0.90^{b}	1.63^{a}	9.08^{a}	28^{a}

Root Dry Weight

Elfasher provenance recorded significantly bigger root dry weight of the provenances. Root dry weight values ranged from 1.77 to 0.69 g. The least root dry weight was recorded by Bara provenance and this was significantly different from the rest of the provenances which shown no significant differences as showed in Table.3.

Shoot Dry Weight

The provenances of Elobied, Elfasher, Senga, Abassya and Eldalang showed no significant differences between them, but were significantly bigger of the provenances. They recorded (1.39, 1.26, 1.25, 1.23, 1.17 g), respectively. Bara provenance recorded significantly lower shoot dry weight the other provenances and recording only (0.67 g). However, the provenances of Eldalang and Gedaref were intermediate with no significant differences between them Table 3.

Shoot / Root Fresh Weight Ratio

Elobied provenance recorded significantly bigger shoot/root ratio (1.7). However, Eldalang and Senga provenances ranked in the second place with significant differences between them recording values of (1 and 0.9) respectively. Abassya and Bara provenances recorded significantly lower values than the other provenances (0.7) as shown in Table 3.

DISCUSSION

Neem tree is a well know multipurpose tree species. It is assumed that it possesses better medicinal and bioactive properties than its close relatives like *A. excels*a and *A. siamensis*. For fuel wood production the neem tree has been identified as one of 223 species suitable for arid and semi-arid regions and one of 145 species suitable for the humid tropics (Pliske, 1983).

It is well documented that neem in its natural range is adapted to drier habitats, but in introduction areas its acclimatization is apparent in both drier and moist climatic regions (Arora, 1993).

Plant population's exhibit genetic variation on several eco-geographical levels and such variation exist along ecological gradients in latitude, altitude and humidity (Hill *et al*., 1998). So, many specialized populations have adapted to certain eco-climatic conditions

(Kundu and Tigersledt, 1997). Therefore, it is always advisable to identify the best seed sources adapted to different ecological regions and to breed them for efficient uses. The results of the present work seem to be insufficient to evaluate neem growth performance as it was limited to only one site and for a very limited time and at the nursery stage. However, it can give some indicators for further comprehensive provenances testing in different eco-climatological sites throughout the country.

In the present work it seems that neem tree, which was supposed to have a narrow genetic base in the Sudan, have fully naturalized in different eco-climatic habitats in the various parts of the country. They exhibited significant variations especially in root length and root collar diameter variables, showing its great adaptability to different edaphic and moisture regimes of the different climatic zones of the Sudan. It has been shown by the results showed seedlings of provenances from Western Sudan have generally bigger RCD as compared to the remainder of the provenances from Central and Eastern Sudan. It is important to ascertain here that Elfasher provenance is the most adapted provenance as it was significantly superior in RCD, and root length. These two variables seem to be crucial for survival in arid and semi-arid conditions because that the seedlings were sturdier and the root system can penetrate longer deep in the soil for moisture even in the drier season. On the other hand, Senga provenance seemed more adapted to higher moisture conditions thus producing longer shoots compared to studied or other provenances. The Gedaref provenance in this trial seems to be the least in most of the growth attributes examined. It has been suggested by (Kundu *et al.*, 1998) that eco-climatic attributes play an important role in the differentiation of neem populations and thereby affect their growth during the early growth stages. Based on these agreements we may attribute the least growth performances of Gedaref provenance to its inability to adapt to the cumulative environmental conditions prevailing there perhaps including the heavy cracking clay soils of the area.

Generally, neem as a species, represented the seven provenances tested can be classified as adaptive species that was able to naturalize in vast areas of the different eco-climatic zones of the Sudan. However, more comprehensive provenances trials of neem are indispensable to facilitate drawing more definitive results on its adaptive capabilities.

CONCLUSION

Differences in growth between provenances show the successful adaptation of plants. Therefore, it is always advisable to identify the best seed sources adapted to different ecological regions and to breed them for efficient uses. From the results of the present work and based on several comments and observations furnished by researchers in the field of ecology, it can be concluded that neem tree, which was supposed to have a narrow genetic base in the Sudan, have fully naturalized in different eco-climatic habitats in the various parts.

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