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Effect of Shade on Xylem Development of *Moringa oleifera* (Alrawag) Seedlings

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Abstract: This study investigated the effect of shade as a moderator of light intensity and temperature on xylem development of seedlings of *Moringa oleifera*. Three levels of shade were used: high shade, medium shade and no shade. Four sequential harvests were carried out monthly for the seedlings starting from four weeks after seed sowing. Cross sections were prepared from the lower and middle parts of the stems at each harvest and vessel volume fraction, vessel diameters, number of vessels and volume fraction of lignified cells were measured. The results showed that the effect of shade was significant on the anatomical variables. Generally, the values of the anatomical variables of the stem wood of the seedlings decreased with increased shade level. The findings indicate the importance of shade to *M. oleifera* seedling development and that it should be considered in nursery conditions.

Key words: *Moringa oleifera*; seedlings**;** wood anatomy; shade levels

INDRODUCTION

Moringa oleifera (Moringa) is a well-known and cultivated species of the thirteen species of the family Moringaceae and one of the world most useful plants (Fahey 2005). It is native to the sub-Himalayan tracts of India, and it has become naturalized in many locations in the tropics and in Africa (Fahey 2005). It is known by several names but is popularly called the "drumstick tree" for its fruits that are used by drummers and the

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"horseradish tree" for the flavour of its roots (Palada and Chang 2003). Moringa is drought resistant, though under drought conditions it may lose its leaves but it recovers them when it rains (Von Mydell 1986). It grows well in areas receiving annual rainfall amounts that range from 250 to 1500 mm (Amaglo 2006). It survives in a temperature range of 25°C to 40 °C, but it can tolerate temperature of 48°C and light frosts. It grows best in neutral to slightly acidic soil and in well-drained loam to clay-loam (Von Mydell 1986).

Moringa has multiple uses that have attracted the attention of researchers, development workers and farmers. It is becoming a source of nutrition, where most of the world's poor people live (Palada and Chang 2003). In Sudan, It is an important, widely- distributed species (Elamin 1981). It was introduced as an ornamental tree during the British rule (Jahn *et al*. 1986). Due to its many uses, it was planted in the tropical belt of Africa. In Sudan, it is called "Shagarat Alrawag" as the seeds are used for water clarification. It can be cultivated cheaply at the household level or in small communal nursery (Dishna 2000).

Seedling growth of forest tree species is generally affected in the early stage by moisture, light intensity, temperature and other factors (Bonner 1984). Mohamad (1986) reported that seedlings of *Shorea materialis* grown at 30% and 50% light intensity have tall and slim shoots with large dark green leaves and relatively poor root system. These seedlings are unhardened and succulent and are generally sensitive to desiccation. Jahn *et al*. (1986) reported that all seedlings from *Moringa* species grew poorly and succumbed at an early stage of development for unknown reasons.

Further, they indicated that germination and growth of *M. oleifera* seedlings is affected by light and recommended half shade for optimum seed germination. Also, Ahmed (2010) indicated that seedlings growing under high shade were soft and succulent and that indicates poor xylem development. Effect of external environmental factors on xylem development is not fully studied under dry conditions in Sudan, especially the effect of light intensity and temperature. In addition, little is known about the anatomical correlates of the great diversity seen at the morphological level in dry tropical plants (Olson and Carlquist 2000).

Despite the valuable uses of *M. oleifera*, very little is known about its response to light intensity. In nursery practice, it is important to have suitable light conditions to produce well balanced and hardened seedlings suitable for out planting. Thus, the general objective of this study was to investigate the effect of shade, as a moderator of light intensity, and temperature on xylem development of *M. oleifera* seedlings. The specific objective was to study the effect of shade on vessel volume fraction, vessel diameter, number of vessels per mm^2 and volume fraction of lignified cells of seedlings.

MATERIALS AND METHODS

The study was conducted in a site near the nursery of the Faculty of Forestry, University of Khartoum. After clearing, the site was divided into three square units (1.5 x 1.5 m). Each unit was then assigned randomly to one of the three studied treatments, which were high (≈80% shade), medium (≈50% shade) and no shade (≈0% shade). Green nets on the top and the four sides were used to give the required shade; one green net layer was erected for medium shade, double green net layer for high shade and without net for no shade.

Seeds were extracted from fruits of *M. oleifera* that were collected from trees in the study site. Hundred seedlings were raised per treatment in polythene bags (20 x 10 cm, when flat) filled with clay soil (2.75 kg per bag) and surface-irrigated every other day by applying similar amount of water.

Five seedlings were randomly taken from each of the three shade treatments after 1, 2, 3 and 4 months from the date of sowing (four harvests) to prepare microscope slides. Cross sections were prepared from the lower and middle parts of the stems. The sections were put in 50% alcohol for one minute, then transferred to Safranin (0.2 g/100 ml 50% alcohol), left for 10 minutes (to be fixed at the lignified cell walls) and washed in 50%, 70% and 96% alcohol. The sections were transferred to light green stain (0.2 g/100 ml alcohol 95%) for one minute, washed in 96% alcohol and in absolute alcohol and finally transferred to xylol. Each section was mounted in a drop of Canada balsam on a clean slide, then covered with a cover slip and labeled.

Photographs of the slides for the stem wood cross-sections were taken using a 'Moticam 1000' digital camera mounted on an 'Olympus CH20' microscope using a 10X magnifications. The images were printed on A4 paper. Stereological count was conducted following the procedure of Ifju (1983). A sixteen-point grid (3×3 cm in dimension) was drawn on a paper and photocopied on a transparent paper. The grid was fixed on each of the photographs to obtain the point counting (P_P), the number of points of intersection with boundaries generated per unit length of test lines (P_L) and the number of objects or feature in vessels in a contained area of microstructure (N_A). A glass scale was projected through the microscope and the calibration was made to determine the total stereological area. For each shade treatment, 5 slides (one from each of the 5 sample seedlings) per harvest were used and four fields from each slide were examined.

The stereological data were used to calculate the following anatomical variables according to Ifju (1983) equations:

1) Volume fraction for vessel and lignified cell:

$$CF = PPL + PPW$$

where: CF = Volume fraction of vessel or lignified cell; PPL = Volume fraction of vessel lumen or lignified cell lumen; PPW = Volume fraction of vessel cell-wall or lignified cell cell-wall.

2) Horizontal cell diameter of vessel elements:

$$CD\ (H) = P_{L(H)} / 2N_A$$

where: CD (H) = Horizontal cell diameter of vessel elements; $P_{L(H)}$ = Number of intersection points (4x4) of the four horizontal lines of test grid with boundaries of vessels lumen area; N_A = Total number of objects vessels which exist in the total area of the grid.

3) Vertical cell diameter of vessel elements:
$CD\ (V) = P_{L(v)} / 2N_A$

where: CD (V) = Vertical cell diameter of vessel elements; $P_{L(v)}$ = Number of intersection points of the four vertical lines of test grid with boundaries of vessels lumen area; N_A = Total number of vessels which exists in the total area of the grid (4x4).

4) Number of vessels is determined by counting the total number of vessels which exists in the total area of the grid.

Data analysis: One way analysis of variance using SAS statistical package (SAS Institute Inc., 1995) was conducted to study the effect of shade on anatomical variables. Treatment means were separated using Duncan's Multiple Range Test, when the effect of the treatment was significant.

RESULTS

The results showed highly significant differences (P=0.0001) among the shade levels in the anatomical variables. The means and results of the Duncan's Multiple Range Test for the difference among shade levels at each harvest are shown in Figures 1, 2 and 3. An example of the difference in wood anatomical structures like vessel number and lignified cells is shown in Figure 4.

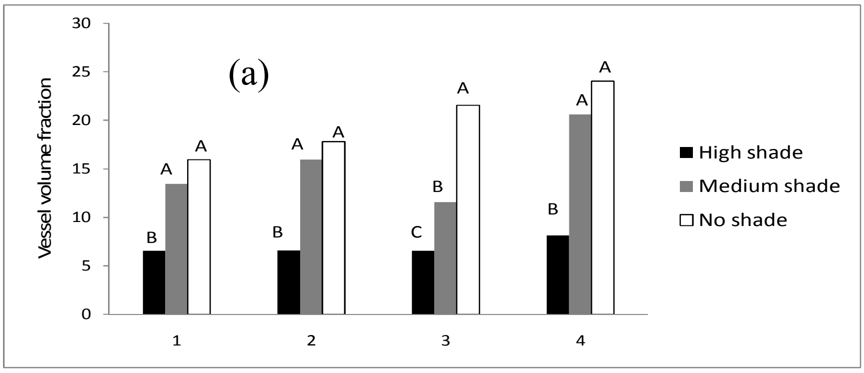
Vessel volume fraction: Effect of shade on vessel volume fraction was highly significant ($P \leq 0.0009$) in all harvests. Generally, there was a decrease in the vessel volume fraction with increasing shade (Figure 1a). High shade resulted in the lowest values in all harvests. The difference between no shade and medium shade was only significant in the third harvest.

Number of vessels: Effect of shade on number of vessel was highly significant (P = 0.0001). Generally, there was a decrease in vessel number with increasing shade (Figure 1b). No shade had the greatest vessel number in the first and second harvest, but the difference was not significant from medium shade in the third and fourth harvests. High shade had the lowest values in the first and fourth harvests.

Horizontal vessel diameter: Effect of shade on horizontal vessel diameter was significant (P ranged from 0.0001 to 0.021). There was a decrease in the horizontal vessel diameter with increasing shade (Figure 2a). High shade had the smallest diameters in all harvests. The difference between no shade and medium shade was significant in the second and third harvests.

Vertical vessel diameter: Effect of shade level on vertical vessel diameter was highly significant (P=0.0001). Also, vertical vessel diameter decreased with increasing shade in all harvests (Figure 3). No shade had the highest values in all harvests. The difference between medium and high shade became significant in the fourth harvest.

Volume fraction of lignified cells: Effect of shade on volume fraction of lignified cells was highly significant (P = 0.0001). The results indicate a decreasing trend in the volume fraction of lignified cells with increasing shade (Figure 4). High shade had the lowest value in all harvests. No shade had greater values than medium shade in all harvests except in the second one.



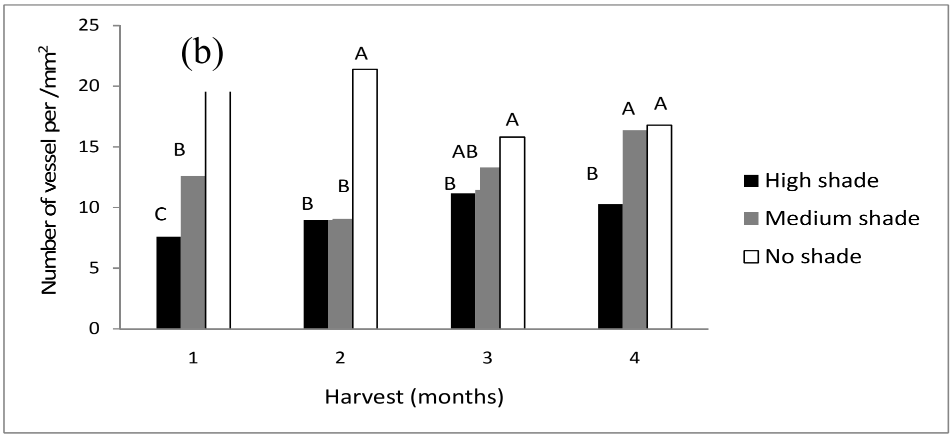


Figure 1. Effect of three shade levels on vessel volume fraction (a) and number of vessels (b) of *Moringa oleifera* on four subsequent seedling harvests (means with the same letter in the harvest are not significantly different, using Duncan's multiple range test)

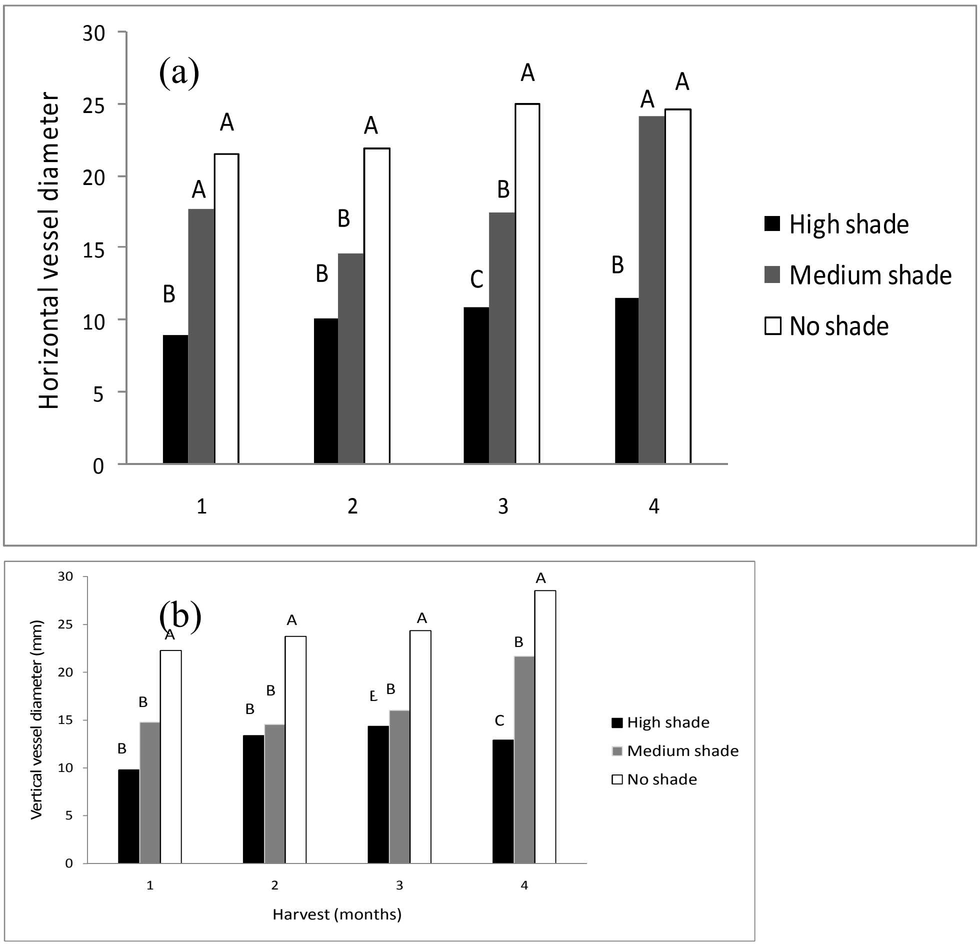


Figure 2. Effect of three shade levels on horizontal vessel diameter (a) and vertical vessel diameter (b) of *Moringa oleifera* on four subsequent seedling harvests (means with the same letter in the

same harvest are not significantly different, using Duncan’s multiple range test).

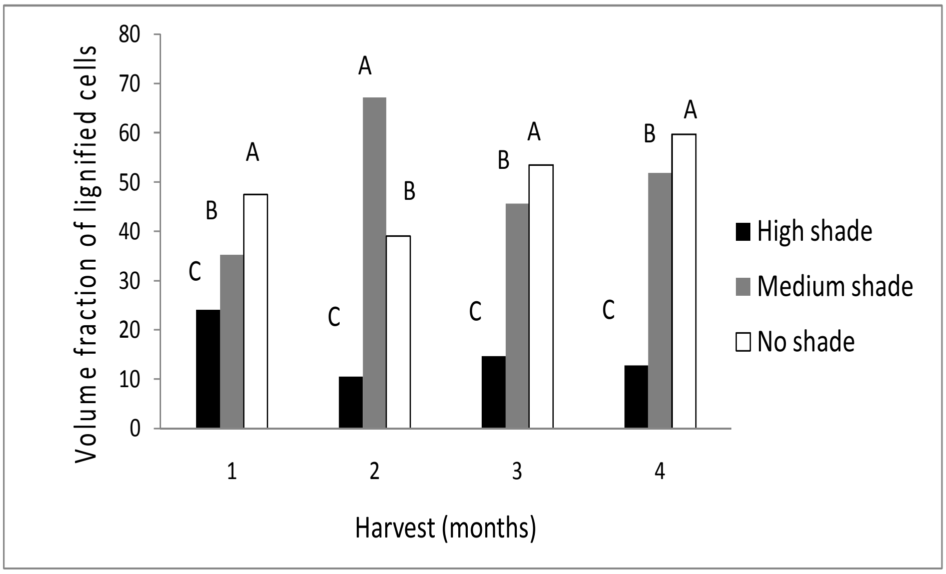


Figure 3. Effect of three shade levels on volume fraction of lignified of *Moringa oleifera* on four subsequent seedling harvests (means with the same letter in the same harvest are not significantly different, using Duncan's multiple range test)

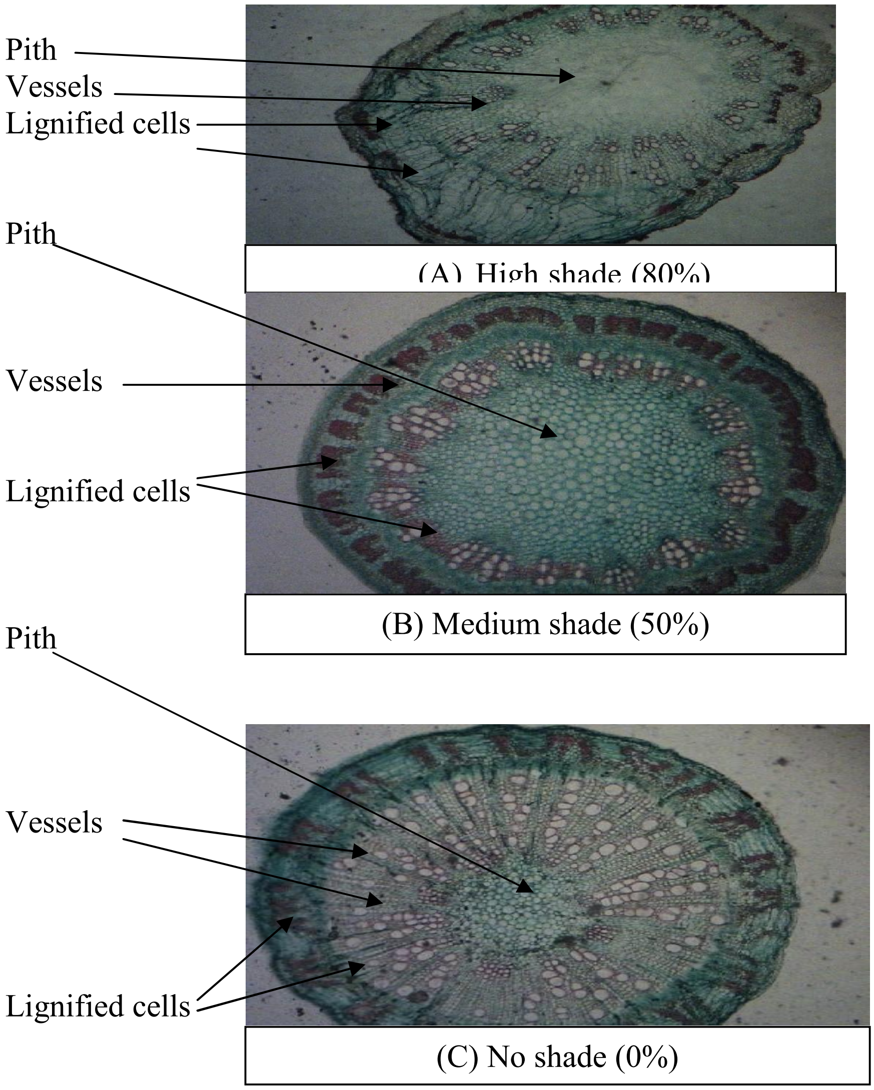


Figure 4. Effect of three shade levels on number of vessels and lignified cells of *Moringa oleifera*, after 12 weeks from seed sowing (third harvest)

DISCUSSION

The results confirmed that shade level (high shade, medium shade and no shade) had significant effect on the stem wood anatomy. Generally, vessel volume fraction, horizontal and vertical vessel diameters, number of vessels and volume fraction of lignified cells decreased significantly with increased shade level.

These results indicate that the shade intensity had a direct effect on the development of the conductive system of the seedlings. The conductive system increased with decreasing shade intensity. This is in line with the expected needs of the seedlings, where seedlings growing under no shade need a more effective conductive system to cope with their more successive growth needs and transpiration demand.

The increase in the number of lignified cells with decreasing shade intensity is in line with the increase in stem hardness with decreasing shade intensity reported by Ahmed (2010). The decrease in volume fraction of lignified cells with increased shade is similar to what was found by Doley (1978), who concluded that the rate of cambial cell division in *Eucalyptus grandis* was approximately 50% greater in the light than in the low light treatments.

This study clearly shows that the xylem during early growth of *Moringa oleifera* seedlings is affected by the level of shade. High shade is associated with lower vessel volume fraction, lower number of vessels, lower vessel diameters and lower volume fraction of lignified cells. These results are in line with the poor growth performance of *Moringa oleifera* seedlings under high shade as reported by Ahmed (2010). On the other hand, the medium and no shade levels, which had the larger sizes and dry weight, are associated with higher number of vessels, higher vessel volume fraction and larger horizontal vessel diameters.

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تأثير الظل على نمو خشب شتول المورنقا (الرواق)

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المستخلص: إستهدفت الدراسة إختبار تأثير الظل كوسيط بين شدة الإضاءة ودرجة الحرارة على تطور خشب شتول المورنقا . تمت دراسة ثلاثة مستويات من الظل وهى ظل عالي ، ظل متوسط ولا ظل. أجريت أربع حصدات متتابعات شهريا للشتول ابتداء من أربعة اسابيع من تاريخ زراعة البذره . جهزت قطاعات عرضية من وسط وأسفل السيقان في كل حصده لقياس نسبة حجم الأوعية ، وأقطار الأوعية ، وعدد الأوعية ، ونسبة حجم الخلايا المتلجننة . اظهرت النتائج وجود تاثير معنوى للظل على المتغيرات التشريحية . عموماً انحفضت قيم المتغيرات التشريحية لخشب الساق فى الشتول مع زيادة مستوى الظل . أشارت النتائج إلى أهمية الظل لتطور خشب شتول المورنقا وضرورة أخذه في الإعتبار تحت ظروف المشتل .

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