Hardening off watering interval of East African greenheart (*Warburgia ugandensis*), nursery seedlings in East Mau watershed, Njoro, Kenya

Abstract

Water is becoming an increasingly scarce resource in most areas but yet essential in establishing nursery seedlings. This calls for the effective and efficient use of this important resource. Hardening off of nursery seedlings through reduction of watering regime is necessary before transplanting seedlings to the field. This leads to better survival yet the interval of watering is not well established and this might vary with species and locality. An experiment was set up during the dry season just before planting out to determine the best hardening off watering interval for East African greenheart (*Warburgia ugandensis*) seedlings in Egerton University, East Mau watershed, Njoro, Kenya. The experiment was laid down as a Completely randomized design (CRD) with 5 treatments replicated 3 times. Treatments comprised of different watering intervals, which were as follows: twice daily, once daily, 2 days, 4 days and 6 days. These treatments were applied for 2 months on 9 months old seedlings during January to March 2018. Analysis of variance was used to determine treatment differences while DMRT was used to determine the significantly different treatment means at $p \leq 0.05$. The results revealed that the best growth was shown by twice daily, once daily, 2 days and 4 days intervals for shoot biomass, total plant biomass and total leaf area. However, the recommended watering interval is 4 days since it showed good growth for most of the variables with minimal water use equivalent to 12.5% of the water used by seedling watered twice daily which saves 87.5% of the water used. The results can be applied in the Kenyan highlands for East African greenheart and other leaf succulent plants. However more studies needs to be done for other non-succulent species using different pot sizes and soil mixtures.

**Keywords:** Nursery seedlings, *Warburgia ugandensis*, watering interval

1.0 Introduction

*Warburgia ugandensis* is commonly known as East African greenheart, a species of evergreen tree native to Africa. It is mainly found growing in Kenya, Ethiopia and some parts of western
Africa. The leaves are succulent and can be used to flavor curries and the extract has been reported to show some antimalaria, antifungal and antibacterial properties (Olila et al., 2001; Were et al., 2010)

Water is an important natural resource that vital for growth of plants, but there is a growing concern on its availability (Goyne & McIntyre, 2003). With the effects of climate change, water is becoming increasingly scarce in most geographical zones globally (Morrison et al., 2009). Previous permanent water supply sources are becoming seasonal hence posing serious challenges to tree nursery establishment and management, especially in the drier regions of the sub-tropics

Plants vary greatly in their response to watering intervals depending on edaphic and environmental conditions. Hardening off operation is essential in the nursery and it involves reducing watering of seedling 4-8 weeks before planting out for the purpose of acclimatization (Inoti, 2001). Changes in water availability can affect seedling resource allocation (Blain and Kellman, 1991) which subsequently affect water uptake and photosynthesis, hence modifying growth and survival of field planted seedlings. An experiment was therefore set up 8 weeks before planting out to determine the best watering interval for Warburgia nursery seedlings during hardening off in the eastern Mau catchment of Kenya.

Early growth of seedlings mainly depends on food reserves contained in the cotyledons and also soil moisture availability, but after depletion of food reserves, seedlings rely on manufactured photosynthetic reserves for their continued growth and survival (Bargali and Tewari, 2004). Soil moisture plays a key role in nutrient uptake from the growing media to support growth (Shao et al., 2008). Various vital processes in plants such as cell division, cell elongation, stem as well as leaf enlargement and chlorophyll formation depend on plant water availability.

Regular watering is therefore ideal for nurseries to produce high quality seedlings (Inoti, 2001 and Simon et al., 2011) at profitable levels since any stagnation in growth or subsequent mortality translates into economic loss to a nursery owner. Low water supply can lead to huge losses because seedlings take long to reach an appropriate size (30 cm height) for grafting (Mhango et al., 2008) and planting out or for sale.
Water is the main constituent of living organisms and it is involved in photosynthesis process thus its availability at tolerable interval affects productivity of most plants. According to Hartmann et al. (2005), water stress decreases the growth of plants in terms of leaf number, leaf area, and biomass weight and stomata conductance. Root growth is more preferred over foliage in water scarce environments and extensive rooting is a special adaptation for plant survival in arid areas (Abbott, 1984; Inoti, 2018).

Water is an important factor in dry land forestry and it is critical to tree growth and development in the tropics (Awodola and Nwoboshi, 1993). According to Miller et al. (1999), for each ton of vegetative growth, hundreds of tons of water may be consumed by the growing plant especially in dry sites. As observed by (Awodola, 1984), the reduction in relative water contents affects physiological processes and hence plant growth. Earlier works by Huang et al. (1985) reported that root to shoot ratio to be 3.5 times higher in water stressed plants compared to non-stressed ones.

2.0 Research methodology

2.1 Study site

The study was conducted at Agroforestry tree nursery, Egerton University, Njoro, Kenya, within the eastern Mau watercatchment. The University is located in Njoro, a small community approximately 25 kilometres southwest of the town of Nakuru. This is located approximately 182 kilometres, by road, northwest of Nairobi. The study site lies on a latitude 0°22'11.0"S, Longitude 35°55'58.0"E and an altitude of 2,238 m above sea level. The area falls in agro ecological zone Lower Highland 3. The experimental site receives mean annual rainfall of 1200 mm while the distribution of rain is bimodal with long rains between April and August and short rains between October and December yearly. The temperatures lie between 10.2 and 22.0°C (Ngetich et al., 2014) while the soils are mollic andosols (Kinyanjui, 1979). The experimental period received low rainfall especially during January and February (11.5 mm) which were coupled with moderately high temperatures (21.9 °C) (Table 1).

Table 1: Rainfall and temperature received in the study site during the experimental period (January to March 2018)
<table>
<thead>
<tr>
<th>Months/parameters recorded</th>
<th>January 2018</th>
<th>February 2018</th>
<th>March 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total rainfall (mm)</td>
<td>11</td>
<td>12.3</td>
<td>194</td>
</tr>
<tr>
<td>No. of rainy days</td>
<td>3</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Average temperature (°C)</td>
<td>21.2</td>
<td>22.6</td>
<td>19.6</td>
</tr>
<tr>
<td>Maximum temperature (°C)</td>
<td>22.9</td>
<td>23.7</td>
<td>22.3</td>
</tr>
<tr>
<td>Minimum temperature (°C)</td>
<td>17.6</td>
<td>21.0</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Source: Department of Water Engineering, Egerton University, Njoro

2.2 Experimental design
The experiment was laid down in a Completely randomized design (CRD) with 5 treatments replicated 3 times. Treatments comprised of different watering intervals, which were as follows: twice daily, once daily, 2 days, 4 days and 6 days. Forest soil was used as the potting media while the polythene pot size was 9x20 cm, in width and length respectively. The seedlings were raised in the nursery for 9 months before being randomly selected for the experiment. Each treatment consisted of 10 plants per replicate which were then raised in the nursery for 2 months, out of which 7 seedlings were randomly sampled for assessment. The experiment was carried out between Mid January to Mid March 2018, during the dry period.

The variables measured included; height, root collar diameter, 3rd internode length, number of leaves, leaf length and width, leaf area, seedling sturdiness quotient, root length, plant and root biomass. Seedling sturdiness quotient (SSQ) = shoot height (cm) divided by root collar diameter (mm). The smaller the quotient the better the sturdiness.

2.3 Data analysis
Data of the measured variables was subjected to statistical analysis using ANOVA model procedures of Genstat statistical package (2013). Variations between the treatment means were compared using Duncan’s Multiple Range Test (DMRT) at P ≤ 0.05. In addition, coefficient of variance (CV %) was calculated to reveal the relative measure of variation that existed within the data.
3.0 Results and discussion

Seedling growth was relatively uniform for all the treatments ranging from twice daily to 4 days interval. However, the 6 days watering interval compromised the growth for most of the shoot, foliage and root variables measured. According to Levy and Krikum (1983), low water levels in plants below a critical level usually triggers changes in all structures sometimes leading to the death of the plants. This is further supported by earlier observations by Awodola (1984) and Farah (1996), who reported that water scarcity reduces growth, yield and other physiological processes.

3.1 Effect of watering interval on shoot growth of Warburgia ugandensis nursery seedlings

Results showed that shoot biomass and total plant biomass for twice daily, once daily, 2 days and 4 days interval were significantly ($P < 0.05$) higher compared with 6 days interval (Table 2). These findings corroborate with studies by Abo El-Khei (2000) who reported that water stress can lead to low shoot dry weight. Water deficiency imposes huge reductions in crop yield through diminished leaf carbon fixation and general growth inhibition (Chaves & Oliveira, 2004).

More recent studies by Daba and Tadese (2017) also concluded that Moringa oleifera and Grevillea robusta watered twice daily with 1.5 liters and Cordia africana watered twice after one day with 2 liters per plot ensures good growth performance of those tree seedlings species. These findings therefore have implications on water wastage, reduced labor costs and maximizing profitability of tree seedlings production.

According to Olaoye and Oyun (2019), two or three times seedlings watering per week of Terminalia ivorensis, Terminalia superba, Cleistopholis patens and Mansonia altisima is most effective for improving the physiological growth which can enhance the domestication and cultivation of these seedlings in their environment. Excessive water encourages the growth of microorganisms such as bacteria and fungi which might cause disease in the seedlings. Similarly, Oyun et al. (2010) reported that, watering twice weekly is most suitable for tending the seedlings of Acacia senegal in the nursery. This is evident because daily watering produced fragile and
succulent seedlings that cannot withstand the harsh drought condition in the field. This is also in conformity with the observation made by Awodola (1984) and Huang et al. (1985).

Similarly, height and seedling sturdiness quotient (SSQ) for twice (48 cm and 469 respectively) and once daily (48.5 cm and 480.7 respectively) were significantly higher compared with 6 days interval (44.03 cm and 378.7 respectively) which was the lowest. The lowest SSQ shows the best sturdiness and survival in the field. This tends to decrease with reduction in watering.

Table 2: Effect of watering interval on shoot growth of *Warburgia ugandensis* nursery seedlings

<table>
<thead>
<tr>
<th>Watering interval</th>
<th>Height (cm)</th>
<th>3rd internode length (mm)</th>
<th>Shoot biomass (g)</th>
<th>Total plant biomass (g)</th>
<th>Seedling sturdiness quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 days</td>
<td>44.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.67</td>
<td>8.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>378.70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 days</td>
<td>46.83&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.00</td>
<td>10.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>439.30&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 days</td>
<td>46.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.33</td>
<td>10.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>423.30&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>0nce daily</td>
<td>48.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.33</td>
<td>10.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>480.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Twice daily</td>
<td>48.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.00</td>
<td>10.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>469.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CV%</td>
<td>3.6</td>
<td>12</td>
<td>8.8</td>
<td>9.1</td>
<td>8.1</td>
</tr>
<tr>
<td>SED</td>
<td>1.37</td>
<td>0.97</td>
<td>0.73</td>
<td>1.19</td>
<td>28.93</td>
</tr>
</tbody>
</table>

Means with different superscript letters within a column differ significantly using DMRT at P≤0.05.

3.2 Effect of watering interval on leaf growth of *Warburgia ugandensis* nursery seedlings

Watering twice daily showed the highest number of leaves (16.23) and total leaf area (436.7 cm<sup>2</sup>) which were significantly (P ≤ 0.05) higher compared with all the other treatments except once daily interval (14.93 and 418 cm<sup>2</sup> respectively) (Table 3). On the other hand, 6 days interval showed significantly the lowest total leaf area (289.3 cm<sup>2</sup>) compared with all the other treatments. These findings are in agreement with earlier studies by McMaster and Smike (1988) who explained that during vegetative growth, phyllochron decreases under water stress leading to leaves becoming smaller, which consequently results in low leaf area index.

Table 3: Effect of watering interval on leaf growth of *Warburgia ugandensis* nursery seedlings

<table>
<thead>
<tr>
<th>Watering</th>
<th>Number of leaves</th>
<th>Leaf length</th>
<th>Leaf width</th>
<th>Single leaf area</th>
<th>Total leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 days</td>
<td>1.37</td>
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<td>0.97</td>
<td>0.73</td>
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<td>28.93</td>
</tr>
</tbody>
</table>
However, once daily interval showed the highest single leaf area (28 cm²) which was significantly higher compared with all the other treatments except 2 days interval (27.8 cm²).

Hartmann et al. (2005) reported that water stress decreases the growth of plants in terms of leaf number, leaf area and stomata conductance which is consistent with the current findings.

### 3.3 Effect of watering interval on root growth of Warburgia ugandensis seedlings

Watering once daily showed the highest root collar diameter (9.9 mm) and root length (24.93 cm) which were significantly higher compared with 6 days interval (8.6 mm and 21.33 cm respectively) (Table 4).

**Table 4: Effect of watering interval on root growth of Warburgia ugandensis nursery seedlings**

<table>
<thead>
<tr>
<th>Watering interval</th>
<th>Root collar diameter (mm)</th>
<th>Root length (cm)</th>
<th>Root biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 days</td>
<td>8.60b</td>
<td>21.33b</td>
<td>4.73</td>
</tr>
<tr>
<td>4 days</td>
<td>9.33ab</td>
<td>22.93ab</td>
<td>6.00</td>
</tr>
<tr>
<td>2 days</td>
<td>9.13ab</td>
<td>22.77ab</td>
<td>5.80</td>
</tr>
<tr>
<td>Once daily</td>
<td>9.90a</td>
<td>24.93a</td>
<td>6.67</td>
</tr>
<tr>
<td>Twice daily</td>
<td>9.77a</td>
<td>23.73ab</td>
<td>6.20</td>
</tr>
<tr>
<td>CV%</td>
<td>5.5</td>
<td>7.2</td>
<td>20.5</td>
</tr>
<tr>
<td>SED</td>
<td>0.42</td>
<td>1.36</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Means with different superscript letters within a column differ significantly using DMRT at P≤0.05.

Further results showed that watering twice daily tends to compromise growth of roots in the highlands and this was observed by once daily showing the highest growth for the variables measured though not significant. According to Komer et al. (1999), excess water in plant cells may retard physiological processes. For example, stomata conductance, a numerical measure of the maximum rate of passage of water vapour for transpiration or carbon dioxide through the stomata for photosynthesis is influenced by the soil-water balance (Komer et al., 1999). Simon et al. (2011) reported 2 day watering interval for Persia americana and Vangueria infausta.
showing the highest root collar diameter growth while Daba and Tadese (2017) also recorded similar results with *Cordia africana* after 1 day interval.

Studies by ElHadi *et al* (2013) showed that moderate stress (9 days watering interval) facilitated seedling height development compared to frequent irrigation (3 and 6 days). While longer period (12 days) resulted in negative impact on the seedling development by affecting the water potential. With moderate stress, the carbohydrates are transported to the root system. The root system benefits from this supply by being under less stress compared to the shoot, leading to better growth (Hsiao 1973). Moderate stress also has minute effect in carbon uptake Nitrogen and mineral uptake by the actively growing root system may concentrate in the shoot.

The results of the current research showed that the watering interval of 4 days was the most ideal for Warburgia seedlings raised in the highlands. This is consistent with other recent studies by Inoti (2018) who recommended 4 days interval for jojoba seedlings while Sale (2015) also recommended 3 and 5 days interval for *Parkia biglobosa* in dry areas. Similar work carried out on *Acacia senegal* by Isah *et al.* (2013) indicated that the species performed better when watered once in three days and this reflects its capability to cope with drought stress. More recent studies by Inoti and Cherop (2022) recommended 2 days watering interval in *Prunus africana* seedlings which is a non- succulent species in the Kenyan highlands.

**4.0 Conclusion and recommendations**

Four days watering interval showed good growth with economical water use and this is essential during the period of seedling hardening off. The results can be applied in the Kenyan highlands for *Warburgia ugandensis* and other succulent leaf plants. However more studies needs to be done for other species using different pot sizes and soil mixtures.

**References**


Genstat (2013). General statistical software. Learning and Development Centre Resource. Warwick University, UK.


