Original Research Article

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

Abstract

Water has been ~~is becoming an increasingly~~ a scarce resource in most agriculture areas and ~~but yet~~ essential in the establishementing ~~ing~~ of nursery seedlings. This requires~~calls for the~~ effective and efficient use by farmers of this important resource. Hardening ~~off of~~ nursery seedlings through the reduction of watering regimes is necessary before transplanting seedlings to the field. Hardening~~This~~ leads to better survival but when~~yet~~ the interval of watering is not well established ~~and~~ this might affect~~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, in 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*, bedding plant, watering interval

Formatted: Justified

Formatted: Font: Italic

1.0 ~~1.0~~ Introduction

In Africa, *Warburgia ugandensis* is commonly known as East African greenheart, a native 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 plant growth~~ of plants~~, but there is a growing concern about~~on~~ its availability (Goyne & McIntyre, 2003) in the next future. 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 transplanting with~~out for~~ the purpose of acclimatization (Inoti, 2001). Changes in water availability can affect seedling resource allocation (Blain and Kellman, 1991) because~~which subsequently~~ affect water uptake and photosynthesis, hence modifying growth and survival of field transplanted 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 next~~also~~ from soil moisture availability.~~,~~ A~~but a~~fter depletion of food reserves, seedlings rely on manufactured photosynthetic reserves for ~~their continued~~ growth, development and survivalship (Bargali and Tewari, 2004). Soil moisture is ~~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.

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0 cm + Indent at: 0.63

Formatted: Justified

Formatted: Justified

Regular watering is ~~therefore ideal~~ required under~~for~~ ~~nurseries~~ nursery conditions to ~~produce~~ produce cost effective ~~high quality~~ seedlings (Inoti, 2001 and Simon *et al.*, 2011) because ~~at profitable levels since any~~ the stagnation in growth or subsequent mortality translates into economic losses to ~~a~~ nursery owners. 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~~ transplanting, ~~planting out or for~~ sales~~.~~ all of them,

Formatted: Justified

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

Comment [JW1]: Material and methods – The experiment still need information for reproducibility.

The seeds were collected from ….

2.1 Study site

The investigation~~study~~ was conducted at Agroforestry tree nursery, Egerton University, Njoro, in Kenya, within the eastern Mau water catchment. 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,~~ northwestern ~~of~~ Nairobi. The study site at ~~lies on a latitude~~ 0°22'11.0"SL, Longitude 35°55'58.0"EL and and 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 where~~ile~~ the distribution ~~of rain~~ is bimodal with long period~~rains~~

between April and August and short period~~rains~~ between October and December~~ yearly~~. The temperature~~s~~ ranges~~lie~~ from~~between~~ 10.2 to~~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 ^{0}C) (Table 1).

Table 1: Rainfall and temperature ~~received in the study site~~ during the experimental period (from January to March, 2018)

Months/parameters recorded	January 2018	February 2018	March 2018
Total rainfall (mm)	11	12.3	194
No. of rainy days	3	4	19
Average temperature (^{0}C)	21.2	22.6	19.6
Maximum temperature (^{0}C)	22.9	23.7	22.3
Minimum temperature (^{0}C)	17.6	21.0	15.8
Source: Department of Water Engineering, at Egerton University, in Njoro			

2.2 Experimental design

The experiment was laid down in a c~~C~~omplete~~ly~~ randomized design (CRD) with 5 treatments replicated 3 times. The treatments were~~comprised of~~ different watering intervals~~,~~ which were as follow~~s~~: 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 from~~between~~ Mid January to Mid March 2018, during the dry period.

The dependent variables were~~measured included:;~~ height, root collar diameter, 3rd internode length, number of leaves, leaf length and width, leaf area, seedling sturdiness quotient, root

Formatted: Justified

Formatted: Font color: Red

Comment [JW2]: The manuscript requires sentences with the information of the chemical characteristics of this soil.

Formatted: Font color: Red

Formatted: Font color: Red

Comment [JW3]: Square shape? Semi-piramedy sape. How many cm^{3}.

Comment [JW4]: How much water was supplied to each pot. Without this value, the experiment cannot be published. My question is: Is there another manuscript under evaluation by reviewers?

Formatted: Font color: Red

Formatted: Font color: Red

Comment [JW5]: 270 days

Comment [JW6]: In the

Comment [JW7]: ????????????????

Formatted: Justified, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers length, plant and root biomass. Seedling sturdiness quotient (SSQ) = shoot height (cm) divided by root collar diameter (mm). ~~The s~~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. Furthermore~~In addition~~, the coefficient of variation~~nce~~ (CV %) was calculated to reveal the relative measure of variation that existed within the data.

Comment [JW8]: As a scientific experiment, I suggest printing the statistical model

Formatted: Justified

3.0 Results and discussion

Comment [JW9]: I suggest printing the Anova table as Table 2 and enumerated the following.

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: Effects of watering interval on shoot growth of *Warburgia ugandensis* nursery seedlings

Watering intervals	Bedding plant Height	Third~~3rd~~ internode length	Shoot biomass	Total bedding plant biomass	Seedling sturdiness quotient
	cm	mm	g	g	unit
6 days	44.03[b]	8.67	8.47[b]	13.20[b]	378.70[b]
4 days	46.83[ab]	10.00	10.57[a]	16.37[a]	439.30[ab]
2 days	46.30[ab]	9.33	10.27[a]	16.47[a]	423.30[ab]
0nce daily	48.50[a]	10.33	10.60[a]	17.27[a]	480.70[a]
Twice daily	48.00[a]	11.00	10.87[a]	16.87[a]	469.00[a]
CV%	3.6	12.0	8.8	9.1	8.1

SED	1.37	0.97	0.73	1.19	28.93

Means with different superscript letters within thea columns 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^2) which were significantly ($P \leq 0.05$) higher compared with all the other treatments except once daily interval (14.93 and 418 cm^2 respectively) (Table 3). On the other hand, 6 days interval showed significantly the lowest total leaf area (289.3 cm^2) 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

Watering interval	Number of leaves	Leaf length (mm)	Leaf width (mm)	Single leaf area (cm^2)	Total leaf area (cm^2)
6 days	11.6^{c}	109.30	29.67	24.90^{c}	289.30^{d}
4 days	13.47^{bc}	114.30	32.33	25.77^{c}	347.70^{c}
2 days	13.27^{bc}	116.00	31.00	27.80^{ab}	369.00^{bc}
0nce daily	14.93^{ab}	123.70	33.00	28.00^{a}	418.00^{ab}
Twice daily	16.23^{a}	115.30	32.67	26.90^{b}	436.70^{a}
CV%	8.1	8.1	8	2	8.4
SED	0.93	7.64	2.07	0.44	25.5

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

However, once daily interval showed the highest single leaf area (28 cm^2) which was significantly higher compared with all the other treatments except 2 days interval (27.8 cm^2). 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

Watering interval	Root collar diameter (mm)	Root length (cm)	Root biomass (g)
6 days	8.60[b]	21.33[b]	4.73
4 days	9.37[ab]	22.93[ab]	6.00
2 days	9.13[ab]	22.77[ab]	5.80
0nce daily	9.90[a]	24.93[a]	6.67
Twice daily	9.77[a]	23.73[ab]	6.20
CV%	5.5	7.2	20.5
SED	0.42	1.36	0.98

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

Comment [JW10]: Zero??????

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

Abbott, I. (1984). Emergence, early survival and growth of seedlings of six tree species in Mediterranean forest.

AboElkheir, M.S.A. (2000): Antitranspirant effects on wheat plants grown under two levels of water supply. *Journals of Agric Sc Moshtohor* 38(2): 823 – 832.

Awodola, A. M. (1984): Growth response of some tree seedlings to drought conditions. Msc. Thesis (unpublished). Pp. 13-16.

Awodola, A. M. and Nwoboshi, L. C. (1993). Effect of source of Potassium and frequency of moisture application on growth and macronutrient distribution in seedlings of *Parkia biglobosa*. *Nigerian Journal of Forestry* 23 (2).

Bargali K. and Tewari A. (2004). Growth and water relation parameters in drought-stressed *Coriaria nepalensis* seedlings. *Journal of Arid Environments* 58: 505–512.

Blain, D. and Kellman, M. (1991). The effect of water supply on tree seed germination and seedling survival in a tropical seasonal forest in Veracruz, Mexico. *Journal of Tropical Ecology* 7: 69-83.

Chaves, M.M. and Oliveira, M.M. (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany* 55: 2365–2384.

Daba, M.H. and Tadese, A.E. (2017). Estimation of Optimum Water Requirement and Frequency of Watering for Different Tree Seedlings at Bako Agricultural Research Center Nursery Site. *Journal of Health and Environmental Research* 3(6): 90-97.

Elhadi, M.A; Ibrahim, K.A. and Magid, T.D.A. (2013). Effect of Different Watering Regimes on Growth Performance of Five Tropical Trees in the Nursery *JONARES* 1:14-18.

Farah, S.M. (1996). Water relations and water requirements of wheat. Gezira Research Station Report. P.O. Box 126, Wad Medani, Sudan. Pp. 24-36.

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

Goyne, P.J. and McIntyre, G.T. (2003). Stretching water-Queensland's water use efficiency cotton and grains adoption program. *Water Science and Technology* 48(7): 191–196.

Hartmann, T; College, M. and Lumsden, P. (2005). Responses of different varieties of *Lolium perenne* to salinity. Annual Conference of the Society for Experimental Biology, Lancashire.

Hsiao, T.C. (1973). Plant responses to water stress. *Annu. Rev. Plant Physiology* 24:519-570.

Huang, R.S; Smith, W.K. and Yost, R.S. (1985). Influence of vesicular arbiscular mycorrhiza on growth, water relations and lead orientation in *Leucaena leucocephala* (Lan) wit. *New Phytology* 99: 229–243.

Inoti, S.K. (2001). Tree nursery management: a reference manual for the tropics. Egerton University press. 34pp.

Inoti, S.K. (2018). Effect of watering interval on the growth of jojoba (*Simmondsia chinensis* L.) seedlings in the drylands of Kenya. *Researchjournali's Journal of Forestry* 5(1): 1-8.

Inoti, S.K. and Cherop, D. (2022). Appropriate watering interval for *Prunus africana* nursery seedlings in Egerton University, Njoro, Kenya. *International Journal of Scientific Research Updates* 03(01): 034–039

Isah, A.D; Bello, A.G; Maishanu, H.M. and Abdullahi, S. (2013). Effect of Watering Regime on the Early Growth of *Acacia Senegal* (LINN) Willd. Provenances. *International Journal of Plant, Animal and Environmental Sciences* 3: 2-9

Kinyanjui, H.C. (1979). Detailed soil survey of Tatton farm, Egerton College, Njoro. Ministry of Agriculture- National Agricultural Laboratories, Nairobi, Kenya.

Komer, C; Scheel, J.A. and Bauer, H. (1999). Maximum leaf diffusive condudtance in vascular plants. *Photosynthetica* 13: 45-82.

Levy, Y and Krikum, J. (1983). Effects of irrigation, water and salinity and root-stock on the vertical distribution of vesicular arbuscular mycorrhiza on citrus roots. *New Phytology* 15: 397-403.

McMaster, G. S and Smike, D.E. (1988). Estimation and evaluation of winter wheat phenology in the Central Great Plains. *Agricultural and Forest Meteorology* 3: 1-18.

Morrison, J; Morikawa, M; Murphy, M; Schulte, P. (2009). Water scarcity & climate change: Growing risks for businesses and investors. A Ceres Report, Ceres, Boston.

Ngetich, K.F; Mucheru-Muna, M. and Mugwe, J.N. (2014). Length of growing season, rainfall temporal distribution, onset and cessation dates in the Kenyan highlands. *Agricultural and Forest Meteorology* 188: 24-32.

Olaoye, B.A. and Oyun, M.B. (2019). Early growth of selected indigenous tree species in response to watering regime. *Tropical Plant Research* 6(2): 192–198

Olila, D; Olwa-Odyek and Opuda-Asibo, J. (2001). Anti-bacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. *Afr. Health Sci.* 1(2): 66-72.

Oyun, M. B; Adeduntan, S. A. and Suberu, S. A. (2010). Influence of watering regime and mycorrhizae inoculations on the physiology and early growth of *Acacia senegal* (L.) Wild. *African Journal of Plant Science* 4(7): 210-216.

Sale, F.A. (2015). Evaluation of watering regimes and different pot sizes in the growth of *Parkia biglobosa* (Javq) Benth seedlings under nursery condition. *European Scientific Journal* 11(12): 313-325.

Shao, H.B; Chu, L.Y; Jaleel, C.A. and Zhao, C.X. (2008). Water-deficit stress induced natomical changes in higher plants. *Comptes Rendus Biologies* 331: 215–225.

Simon, A. M; Festus, K. A.; Gudeta, S; Oluyede, C. A; Betserai, N. and Ramni, J. (2011). Water application rate and frequency affect seedling survival and growth of *Vangueria infausta* and *Persea Americana*. *African Journal of Biotechnology* 10(9): 1593-1599.

Were, P.S; Kinyanjui, P; Gicheru, M.M; Mwangi, E. and Ozwara, H.S. (2010). Prophylatic and curative activities of extracts from *Warburgia Ugandensis* Sprague (Canellaceae) and Z*anthoxylum usambarese* (Engl.) Kokwaro (Rutaceae) against plasmodium knowlesi and plasmodium berghei. *Journal of Ethnopharmacology* 130(1): 158-162.