Drought Stress Modulation by Biochar and Effects on Soil and Performance of Seedlings of Urban Forest Tree Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAA conducted most of the greenhouse experiments and laboratory analyses as part of his Doctorate research. He was responsible for most of the manuscript preparation. Author ASO is the major supervisor, he project conceptualization, provides major insight into the outcomes of the experimental outputs, and helped with manuscript review and editing. Author ASA helped in project management, data analysis, and graphics, manuscript review and editing. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: This study was designed to examine the effects of watering regime and biochar on soil properties and performance of seedlings of urban forest tree species (UFTS) in the nursery.
Study Design: The experiment was a 5 by 3 by 2 factorial scheme involving urban forest tree species, watering regimes and biochar amendment or not.
Place and Duration of Study: seedlings of five UFTS were raised in the Nursery and Screenhouse of Wesley University, Ondo, a rainforest zone of Nigeria.

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Methodology: Seedlings of five Urban Forest Tree Species (UFTS) were subjected to watering at 80, 60 and 35% field capacity (FC) with or without biochar amendment. UFTS evaluated are: *Bauhinia monandra, Delonix regia, Terminalia catappa, Dypsis lutescens* and *Veitchia merrillii.*

Results: Watering regime and biochar amendment exerted significant effects on soil physical and chemical properties, physiological attributes and biochemical constituents and performance o the UFTS evaluated. Watering at 60 and 35% FC increased bulk and particle densities but reduced significantly ($P<.05$) soil moisture content at field capacity compare with watering at 80% FC. Further, the 60 and 35% FC watering exhibited low N, available K^+, Ca^{2+} and Mg^{2+}. Addition of biochar to the variously watered soil considerably reduced bulk density but remarkably increased porosity, field capacity moisture and plant available moisture. Biochar amendment increased soil pH, total and volatile organic matter contents, available K^+ and Ca^{2+}, extractable Mg^{2+} and dissolved phosphate (PO_4^{3+}). The responses of growth traits and biochemical constituents of UFTS to watering regimes was species specific. Relative to 80 % FC watering, seedling growth attributes reduced significantly under deficit water application (60 and 35% FC) in addition to remarkable accumulation of osmolytes (osmoprotectants) and enzymatic activities. Biochar amendment enhanced accumulation of osmolytes and activities of superoxide dismutase, guaiacol peroxidase and catalase enzymes of UFTS seedlings.

Conclusion: Differential watering and biochar amendment affected soil physical and chemical properties and growth of UFTS seedlings evaluated. Biochar amendment of the variously watered soil enhanced seedling growth, and appear as effective strategy for improving soil properties and UFTS performance, and for mitigation of adverse effects of suboptimal watering.

1. INTRODUCTION

Urban forest trees species (UFTS) constitute an important element in the cityscape because their natural graceful shapes make scenic views more spectacular and create window effect [1]. Many UFTS are purposely planted for providing various benefits ranging from ecological, recreation and social functions including [2] to decoration of public places like; schools, events and club centres, hotels, banks, palace, markets, fuel stations etc. These tree species play major roles in microclimatic amelioration especially of urban heat island effect [3] and ultimately decrease the energy needed for cooling buildings [4]. They also act as noise filters and air purifier through capturing particulate matter, carbon dioxide, ozone and other air pollutants originating from traffic and industrial activities [5], thus improves human life quality. Little wonder urban dwellers in Nigeria love to live and work in green environment, thus, cultivate various UFTS around their houses [6].

Urban forest tree species (UFTS) are faced with the challenges of sub-optimal soil moisture availability resulted from inadequate rainfall, reduced infiltration, intermittent seasonal change [comprising short (August break) and long term dry season (December to April)] and common irregular watering among horticulturists in Southwest Nigeria. Mustafa et al. [7] observed a steady decline in the number of raining days, sharp decline in relative humidity and rise in temperature (minimum) in Southwest, Nigeria which constitutes a major environmental stress that hampers survival, establishment, growth and productivity of ornamental plants [8]. Suboptimal soil moisture level also diminished nutrients and water uptake, leaf water potential, growth, net photosynthetic rate, transpiration and stomata conductance in plants [9,10] by triggering over-excitation of the photosynthetic pigments in the antenna, leading to excessive reactive oxygen species (ROS) formation in the chloroplasts, destroyed the organelles membrane structure and increase malondialdehyde lipid peroxidation [11,12]. To combat such oxidative stress, plants have developed strong antioxidants defense system comprising enzymes like superoxide dismutase, peroxidase, catalase etc. and non-enzymatic compounds like proline, soluble sugars, ascorbic acid, carotenoids, phenolic acids and flavonoids etc. However, effectiveness of antioxidant defense mechanisms depend largely on plant species and duration of water stress.

Amendment of soil using biochar has been suggested as a viable tool for increasing soil water and nutrients retention capacity, thus, improves plants water use efficiency and productivity [13,14]. Biochar is a human-produced fine-grained and porous substance, generated via pyrolysis from the heating of biomass-derived feed-stocks under oxygen-

limited conditions and deliberately added to soil to improve soil health and sequester carbon [15]. With biochar addition, previous studies had reported enhanced growth and yield of crops [16,17] and reduced frequency and amount of irrigating water needed to grow plants under stress condition [18]. Few studies had evaluated the change in soil properties, growth attributes and biochemical constituents of urban forest trees species (UFTS) under watering regimes while very few studies focused on using "industrially produced" biochar for boosting nursery production of UFTS in urban landscapes of the tropics. This research therefore assessed the changes in the physical and chemical properties of the studied soil, growth attributes and biochemical constituents of the selected UFTS due to suboptimal watering regimes and biochar amendment.

2. MATERIALS AND METHODS

2.1 Treatments and Experimental Design

Sawdust biochar was produced using local reactor at 450 – 500°C and the resident time was 5 hours. Five urban forest trees species (UFTS) namely; *Bauhinia monandra* Kurz, *Delonix regia* (Bojer ex Hook) Raffin, *Terminalia catappa* L., *Dypsis lutescens* (H. Wendl.) Beentze and J. Dransf. and *Veitchia merrillii* (Becc.) H. E. Moore were selected based on their prevalence in Ondo, Nigeria. These tree species were raised at one seedling per polythene bag for one month in the screen house of Wesley University, Ondo, Nigeria. Thereafter, the seedlings were transplanted into perforated plastic pots (Upper × Lower diameter × Height = 26 × 20 × 30 cm) filled with either 6Kg of top soil alone or 5.76Kg of soil mixed with 240g sawdust biochar (equivalent 4% biochar amendment) at the rate of one seedling per pot and watered to field capacity for another one month to ensure full acclimatization and healthy growth.

The treatments were 5 x 3 x 2 factorial combinations of five UFTS, 3 levels of watering and sawdust biochar amendment or not. The pots arranged in complete randomized block design were fully watered on the day of onset of experiment, and weighed. Thenceforth, each pot with plant was weighed in 3-day intervals to determine the evapo-transpiration water loss (ETc). For treatment without biochar application, the pots were either irrigated with the amount of water sufficient to compensate for 80% (optimal watering or control) or 60% (mild watering) or 35% (severe watering) of field capacity (FC).

Each pot in biochar amendment regimes contained 240 g of biochar in addition to 5.76Kg of soil and watered to either 80% FC (optimal watering + biochar), or 60% FC (mild watering + biochar) or 35% FC (severe watering + biochar). The water was delivered slowly to plastic container using a plastic pipette to ensure all the water was captured by the potting mix. The urban forest trees species were allowed to grow for seven months under close monitoring.

2.2 Soil Analysis

To determine bulk density, rhizosphere sample (soil or soil-biochar mix) was collected by inserting a Kopecky ring (Diameter = 4.8 cm, Height = 3.0 cm, Volume = 55.0 cm) on each sampling date after careful harvest of UFTS. The Kopecky ring was immediately covered to avoid moisture loss. Additional 2kg of rhizosphere sample was extracted every week for other analysis. All samples were transported into the laboratory in a zip lock plastic bag until use. In the laboratory, the fresh weight of the rhizosphere sample in the Kopecky ring was noted and then oven-dried in a steel crucible at 105°C until constant weight to obtain dry weights. By means of the samples volume and weight change, the bulk density and particle density of samples was calculated [19]. Soil total porosity was determined from the following formula;

$$TP = 1 - \left[\frac{BD}{PD}\right] \times 100 \qquad (1)$$

From the additional 2Kg fraction, granulometrics of the rhizosphere sample was assessed by hydrometer method. 50g was placed in crucibles of known weight, oven dried overnight at 105°C and the percentage soil moisture content (SMC) was calculated.

$$SMC\ (\%)
= \frac{[\text{Weight of wet sample (g)} - \text{Weight of dried sample (g)}]}{\text{Weight of wet sample (g)}}
\times 100 \qquad (2)$$

200g of the air-dried sample was allowed to saturate by immersing in a dish of water, placed on tripod stand overnight and then transferred into a pre-weighed container (M_1) and the total weight of moist rhizosphere sample and container (M_2) was noted. The sample was oven dried at 105°C until constant weight, and weighed (M_3).

$$WHC\ (\%) = 100 \times \frac{M2-M3}{M3-M1} \qquad (3)$$

Rhizosphere pH was determined by following the principles of Mclean [20]; soil organic matter (SOM) and total organic carbon by adopting loss on ignition method and Walkey-Black method respectively. Total nitrogen in the samples was determined using micro-Kjeldahl method [21]. Atomic Absorption Spectrometry procedures was followed to determine the concentration of available potassium (K), calcium (Ca) and Magnesium (Mg) [22] while available phosphorus was determined colorimetrically following the Brays (No 1) extraction method [23].

2.3 Plant Analysis

Morphological Attributes such as plant height, stem girth, number of leaves, root length and root: shoot ratio were measured using standard methods. The leaf area was determined using Pearcy et al. [24] and total leaf area was estimated. Fresh weights and biomass of leaf, stem and roots (dried at 80 ± 2 °C) was recorded using accurate weighing balance.

2.4 Physiological Variables Determination

Measurements on physiological variables were made on relative water content (turgidity), plant water use and chlorophyll and carotenoids concentrations using standard methods while plant growth rate, relative growth rate, net assimilation rate, leaf area ratio and specific leaf area of the studied seedlings were calculated using standard formulae.

2.4.1 Non enzymatic antioxidants accumulation

Proline content was determined following Bates et al. [25] procedures; total soluble sugars content (TSS) was obtained by using Anthrone method [26]; ascorbic acid and total phenolic acids in the leaf samples were measured respectively by using dichlorophenolindophenol titration method [27] and Folin ciocalteu method at 760 nm using gallic acid as reference [28].

2.4.2 Enzymatic antioxidants activities

From each treatment regime, 0.5g of frozen leaf samples was thoroughly homogenized in 10 ml of ice-cold extraction buffer containing 9 ml 0.2 M potassium phosphate buffer (pH 7.0) and 1 ml 0.1 M EDTA using a pre-chilled pestle and mortar placed on ice. The homogenate was centrifuged at 15,000 rpm for 10 min at 4°C. The supernatant was transferred to a new tube; the slurry was re-suspended in 0.8 ml of the same extraction buffer and centrifuged for 15 min at 15,000 rpm. The combined supernatants were collected as enzyme extract and stored on ice until used.

The photochemical repression of nitrobluetetrazolium (NBT) by superoxide dismutase (SOD) was monitored at 560 nm .The reaction mixture contained 50 μL enzyme extract, 500 μL EDTA (75 mM), 1 mL riboflavin (1.3 μM), 950 μL (50 mM) phosphate buffer, 500 μL methionine (13 mM) and 1 mL NBT (50 μM). Similar reaction mixtures with no enzyme extract was used as blank. The activity of SOD was observed and expressed as SOD IU min-1 mg-1 FW [29]. Catalase activity (CAT, EC. 1.11.1.6) was determined according to the method described by Aebi and Lester [30]. The 3 mL reaction mixture contained 0.1 ml enzyme extract diluted in 2 ml 50 mM potassium phosphate buffer (pH 7.0) and 0.9 ml 10 mM H_2O_2. The decomposition of H_2O_2 was followed as a decrease in absorbance (every 30s for 5 min) at 240 nm. The CAT activity was expressed in terms of mM of H_2O_2 per minute per gram of FW (mMol min^{-1} g^{-1} FW) taking ε = 40 mM^{-1} cm^{-1} as extinction coefficient of H_2O_2 at 240 nm. For guaiacol peroxidase (GPx) activity, the reaction was completed in a 3.0 ml mixture containing 2.7 ml 50 mM potassium phosphate buffer (pH 7.0), 0.1ml 16 mM guaiacol and 0.1 ml enzyme extract. Reaction was initiated by adding 0.1 ml 40 mM H_2O_2 and the oxidation of guaiacol to tetraguaiacol was monitored at 470 nm using spectrophotometer. The change in absorbance was recorded at 15 s interval for 2 min. The GPx activity was estimated as 0.01 unit increase in absorbance due to formation of tetraguaiacol (extinction coefficient ε = 26.6 mM^{-1}cm^{-1}) and presented as mM tetraguaiacol formed min^{-1} mg^{-1} FW [31].

2.4.3 Membrane stability

The level of lipid peroxidation in the membranes was estimated by assessing the malondialdehyde (MDA) content in the leaf of UFTS following the procedure of Hodges et al. [32].

2.5 Statistical Analysis

All experiments were conducted in three replicates and the data obtained on soil and measured values of UFTS were subjected to three ways ANOVA. Treatment Means was separated using the Tukey's Honest Significant Difference (HSD) at P< .05.

3. RESULTS

3.1 Effects of Biochar Amendment and Watering Regimes on Soil Properties

With or without biochar application, watering levels had no remarkable impacts on soil texture (Table 1a). Compared to optimal (control) watering treatment, deficit irrigation (watering at 35% FC) significantly (P<.05) increased rhizosphere bulk density (BD) by 4.29% but slightly reduced particle density (PD) while mild stress (watering at 60% FC) slightly increased both BD and PD. With biochar amendment, optimal (WWB) and mild (MSB) irrigation had considerably reduced BD (~15.0 and 9.7% respectively) and PD (~ 10.44 and 9.23% respectively) whereas severe stress (SSB) caused slight increase in BD and PD. Relative to well watering, total porosity (TP) decreased slightly with mild (60% FC) watering but reduced significantly by ~ 4.73% with severe stress (35% FC watering). In contrast, biochar amendment combination with optimal (WWB) and mild (MSB) watering remarkably enhanced porosity by ~ 16.71 and 10.85% respectively but reduced the same slightly in severe stress (SSB) treatment. Similarly, soil moisture content at field capacity (FC) was remarkably lower by ~ 3.91 and 5.69% under severe and mild stress condition respectively but moisture content at permanent wilting point (PWP) was significantly higher by ~ 3.59 and 4.20% respectively. Both total (TAWC) and plant (PAWC) available water contents decreased significantly by ~ 3.25 and 6.70% respectively under mild stress (60% FC watering) and by ~ 0.99 and 2.30% respectively under severe stress (35% FC watering). Addition of biochar the variously watered soil, field capacity moisture was significantly (P<.05) greater by ~ 14.4, 11.9 and 2.8% respectively for optimal watering (WWB), mild (MSB) compared with severe stress (SSB) conditions whereas PWP reduced drastically by ~ 17.3 and 11.3% with optimal (WWB) and mild stress (MSB) respectively. This resulted in considerable increase in the PAWC (~ 26.3, 20.5 and 4.3% respectively) and TAWC (7.7, 6.9 and 1.9% respectively) of the optimally (WWB), mildly MSB) and severely (SSB) irrigated soil. Soil water holding capacity (WHC) decreased slightly under mild and severe stress conditions but biochar amendment significantly enhanced WHC by 7.4, 8.8 and 4.8% respectively for optimal watering (WWB) and mild (MSB) and severe (SSB) stress conditions.

Relative to well watering treatment, rhizosphere pH was slightly lower by 0.46 and 0.81% respectively while soil electrical conductivity (EC) was slightly lower by ~ 4.1 and 5.7% for the mild and severe stress treatments (Table 1b). In contrast, biochar amendment of soil remarkably raised soil pH by ~ 64 and 56 % and enhanced EC by ~ 26.2, 32.3 and 44.3% respectively for the severe (SSB) and mild (MSB) stress and optimal (WWB) watering conditions. The trio of soil organic matter (SOM), volatile organic matter (VOM) and total organic carbon (TOC) contents decreased slightly with decreasing watering levels (Table 1b) but increased considerably (P<.05) under biochar amendment. Addition of biochar addition enhanced SOM by ~ 5.0, 3.8 and 2.9 , VOM by ~1.60, 1.56 and 1.50 and TOC by ~ 6.3, 5.0 and 3.6 folds for the respective optimal watering (WWB), and mild (MSB) and severe (SSB) stress conditions. In contrast, TOC declined by ~ 18.3 and 20.5% respectively in mild and severe stress treatments. Similarly, mild and severe stress conditions produced ~ 21.5 and 29.1% decline in total nitrogen (N) while biochar amendment enhanced N contents by ~ 2.9, 2.2 and 1.5 for optimal watering (WWB), and mild (MSB) and severe (SSB) stress treatments compared with the non-biochar amendment. The ratio of carbon to nitrogen (C:N) ranged between 12:1 to 13:1 but with biochar amendment, the range was 26:1 to 28:1 with lowest ratio observed under optimal watering and severe stress treatments respectively which indicates increase in C: N ratio with decreasing amount of water applied and that biochar amendment doubled the C: N ratio of soil at all watering levels.

Relative to control, available K^+, Ca^{2+} and Mg^{2+} were slightly lower by ~ 3.6 and 4.1%; 8.9 and 19.0%; and 5.9 and 15.3% respectively for mild and severe stress conditions. Biochar amendment enhanced available K^+ by ~ 14.1, 12.9 and 11.7 percent; available Ca^{2+} was ~ 25.6, 22.9 and 18.4 percent and extractable Mg^{2+} by ~ 6.1, 5.3 and 4.6 percent for the optimal watering (WWB),and mild (MSB) and severe (SSB) stress treatments respectively. Both mild (60 % FC) and severe (35% FC) stress treatments induced slight decreases of 9.7 and 7.1% for extractable phosphate (PO_4^{3+}) while biochar amendment enhanced dissolved phosphate (PO_4^{3+}) by ~ 25.1, 15.7 and 4.5% under optimal watering (WWB), and mild (MSB) and severe (SSB) stress treatments compared with non-biochar amended soil.

Table 1a. Effects of sawdust biochar and watering regimes on soil physical properties

Treatments	Codes	Sand	Clay	Silt	Bulk density	Particle density	Total porosity	Moisture content at field capacity	Moisture content at permanent wilting point	Plant available water content	Total available water content	Water holding capacity
Units		%	%	%	gcm^{-3}	gcm^{-3}	%	%	%	%	%	%
80% FC (Control)	WW	66.608ab	15.442a	17.950a	1.398c	2.059ab	47.233c	27.472d	7.473b	19.998d	34.945c	42.022c
60% FC (Mild)	MS	66.581ab	15.437a	17.982a	1.434abc	2.074a	45.881cd	26.974e	7.625a	19.349e	34.599d	41.698c
35% FC (Severe)	SS	66.365b	15.421a	18.214a	1.458a	2.109a	45.000d	26.399f	7.741a	18.659f	34.140e	40.865c
80% FC + Biochar	WWB	67.025a	15.516a	17.459a	1.189e	1.844c	55.126a	31.438a	6.182d	25.256a	37.620a	45.131ab
60% FC + Biochar	MSB	66.942ab	15.475a	17.583a	1.263d	1.869bc	52.358b	30.736b	6.630c	24.106b	37.367a	45.734a
35% FC + Biochar	SSB	66.492ab	15.430a	18.078a	1.405bc	2.091a	46.981cd	28.234c	7.377b	20.857c	35.611b	44.035b
HSD (P<.05)		0.660	0.095	0.756	0.053	0.190	1.981	0.702	0.116	0.691	0.346	1.699

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance.

Table 1b. Effects of sawdust biochar and watering regime on chemical properties of soil

Treatments	Codes	pH	Electrical conductivity	Soil organic matter	Volatile organic matter	Total organic carbon	Total nitrogen	C:N ratio	Phosphorus	Potassium	Calcium	Magnesium
Units			µS cm^{-1}	%	%	%	%		gkg^{-1}	mgkg^{-1}	mgkg^{-1}	mgkg^{-1}
80% FC (Control)	WW	6.026b	67.667c	4.471d	2.692b	2.039d	0.172d	11.651b	7.417cd	1.258d	1.898d	1.277c
60% FC (Mild)	MS	5.998b	64.917c	4.327d	2.656b	1.665d	0.135d	12.519b	6.893cd	1.213d	1.729d	1.202c
35% FC (Severe)	SS	5.977b	63.833c	3.962d	2.600b	1.622d	0.122d	13.187b	6.696d	1.207d	1.537d	1.083c
80% FC + Biochar	WWB	6.667a	97.606a	22.162a	4.320a	12.864a	0.494a	26.059a	9.280a	17.747a	48.542a	7.770a
60% FC + Biochar	MSB	6.618a	89.500b	17.026b	4.187a	10.135b	0.373b	27.111a	8.578ab	16.201b	43.400b	6.773ab
35% FC + Biochar	SSB	6.583a	85.417b	12.961c	4.044a	7.329c	0.266c	27.848a	7.752bc	14.748c	34.974c	5.846b
LSD (P<.05)		0.557	0.081	4.066	1.352	2.729	0.093	12.873	1.056	1.453	5.142	1.924

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance

3.2 Effects of Watering Regimes and Biochar Amendment on Growth Variables and Biochemical Constituents of UFTS Seedlings

3.2.1 Growth attributes of UFTS

Seedlings of *D. regia* were superiorly taller, thicker with relatively large leaves, deeper root; greater fresh and dry weights, high root: shoot ratio and seedling vigour (calculated using Dickson Quality Index, DQI) which reflects better performance in water stress condition compared to other examined UFTS (Table 2a). Juvenile *T. catappa* exhibited highest number of leaves and total leaf area which bestowed on it better commercial or aesthetic value than all other evaluated UFTS. On the other hand, palm seedlings especially *D. lutescens* showed the lowest values for all the morphometrics except for the thinnest stem girth exhibited by *V. merrilli* seedlings and lowest RSR detected in *B. monandra* seedlings (Table 2a). Net assimilation rate and plant growth rate were higher for semi deciduous species (i.e. *D. regia, T. catappa* and *B. monandra*) than the palm species (i.e. *V. merrillii* and *D lutescens*) with relative growth rate and specific leaf area higher in *D. regia* seedlings than all other examined UFTS (Table 2b). However, relative water content and leaf area ratio were relatively greater in palm species (*D. lutescens* and *V. merrillii*) than semi deciduous species. Chlorophyll content was highest in *V. merrillii* while *T. catappa* and *D. regia* exhibited relatively higher chlorophyll contents than *B. monandra* (Table 2b). The ratio Chl. a/b was highest in *D. lutescens* and lowest in *T. catappa* whereas the highest Car/Chl ratio was observed in *B. monandra* (Table 2b). Water demand and plant water use were highest for *D. regia* but *V. merrillii* exhibited the lowest water demand and plant water use. However, highest and lowest water use efficiency was detected in *T. catappa* and *D. lutescens* seedlings respectively (Table 2b).

3.2.2 Biochemical constituents of UFTS seedlings

Highest contents of ascorbic acid (Asc), total flavonoids (TFC) and total phenolic acid (TPC) were detected in *V. merrillii* which probably led to the lowest MDA content obtained in this species while the lowest TPC, TFC, Asc, proline and total soluble sugar (TSS) was detected in *B. monandra*, a suspected reason for accumulating

the highest MDA content and excessive leaf senescence (Table 2c). *D. regia* seedlings accumulated the highest total soluble sugar and crude protein while *T. catappa* seedlings exhibited greatest accumulation of proline which could have been responsible for higher relative water content (RWC) in *T. catappa* than in *D. regia, B. monandra* and *D. lutescens.* Activities of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPx) was relatively lower in *D. lutescens* than other examined UFTS but *D. regia* exhibited the highest activities of SOD and CAT while greatest GPx activity was observed in *B. monandra* which might enhance drought tolerance in these species than other investigated UFTS especially *D. lutescens.*

3.2.3 Biochar amendment: Growth attributes and biochemical constituents of UFTS seedlings

With biochar amendment, remarkable improvement was detected in majority of the growth attributes of all the examined UFTS such that shoot height, number of leaves, stem girth, total leaf area, plant fresh weight, plant biomass and seedling vigour [measured by using Dickson Quality Index, DQI] increased by ~24.1, 30.9, 17.8, 77.7, 18.7, 17.5 and 10.3% respectively compared to seedlings in biochar-free regimes. However, biochar application caused slight decline (3.4%) in the root: shoot ratio compared to seedlings grown without biochar amendment (Table 3a). Similarly, from Table 3b, biochar amendment remarkably ($P<.05$) augmented the leaf relative water content (~3.9%), leaf area ratio (~12.4%), chlorophyll a (~10.0%), chlorophyll b (~18.9%), total chlorophyll (~11.4%) and plant water use (~17.6%) of UFTS while carotenoids content, ratio of carotenoids to chlorophyll and water use efficiency declined significantly ($P<.05$) with addition of biochar relative to seedlings grown without biochar addition (Table 3b).

Contents of total flavonoids (~17.6%), total phenolic acids (~5.8%), ascorbic acid (~19.5%), total soluble sugar (~11.4%) and proline (~25.2%) were remarkably ($P<.05$) lower with biochar amendment whereas, crude protein content of the tested UFTS increased by ~ 11.1% (Table 3c). At the same time, superoxide dismutase (SOD), guaiacol peroxidase (GPx) and catalase (CAT) were significantly ($P<.05$) up-regulated by ~ 8.3, 17.0 and 38.6% respectively in biochar grown UFTS than in biochar-free regime (Table 3c). In contrast, malondialehyde

lipid peroxidation was significantly (P<.05) depressed by ~ 15.2% in biochar-grown UFTS grown than those grown in biochar-free regimes (Table 3c).

3.2.4 Effects of watering regimes on growth attributes and biochemical constituents of UFTS

On average, water applied and plant (UFTS) water use of UFTS were significantly (P<.05) lower by 28.8 and 30.1% respectively with severe (35% FC) watering treatment and by ~ 13.5 and 9.6% respectively with mild watering treatment. Generally, all the measured growth traits of UFTS seedlings were higher in optimal (80% FC) than in suboptimal (60 and 35% FC) watering condition (Table 4a). In other words, severe (35% FC) watering caused significant (P<.05) reduction in the shoot height (19.1%), stem girth (15.5%), leaf area (29.0%) and total leaf area (49.7%) of UFTS seedlings whereas mild (60% FC) irrigation significantly (P<.05) suppressed the total leaf area (~ 20.2%) of UFTS seedlings compared with optimally (80% FC) watered seedlings. The stem girth of UFTS was remarkably (P<.05) thicker in mild than in severe irrigation. The UFTS exhibited significantly (P<.05) lower chlorophyll a (12.3%), chlorophyll b (18.0%) and total chlorophyll (14.9%) with severe (35% FC) watering. Relative water content (3.3 and 1.9%), plant growth rate (16.4 and 10.0%), relative growth rate (6.3 and 3.7%), leaf area ratio (9.9 and 4.3%) and specific leaf area (31.5 and 9.6%) decreased slightly whereas net assimilation rate (5.0 and 0.4%), chlorophyll a/b ratio (12.6 and 3.3%) and carotenoids/chlorophyll ratio (26.4 and 12.9%), carotenoids (11.8 and 8.1%) and water use efficiency (32.8 and 20.9%) increased slightly in severe (35% FC) and mild (60% FC) irrigation treatment respectively (Table 4b).

Activities of enzymatic antioxidants- superoxide dismutase (SOD), guaiacol peroxidase (GPOx), and catalase (CAT) decreased significantly (P<.05) by ~ 15.5, 25.0 and 62.3% in severe (35% FC) watering condition but mild (60% FC) watering caused considerable (P<.05) decline of 6.7 and 18.3% in GPOx and CAT activities respectively compared with optimally (80% FC) watered UFTS (Table 4c). With severe (35% FC) irrigation, seedlings of UFTS accumulated significantly (P<.05) higher contents of proline (47.6%), total soluble sugar (19.7%), total phenolic acids (37.7%) and total flavonoids (20.6%) but stored remarkably (P<.05) lower contents of crude protein (20.9%) whereas with mild (60% FC) watering, flavonoids content (6.8%) and phenolic acid content (23.8%) were statistically (P<.05) higher but crude protein content (8.5%) was remarkably lower in relation to optimally watered UFTS (Table 4c).

4. DISCUSSION

4.1 Effects of Watering Regime on Soil Properties

The remarkably low total porosity, total and plant available water content and moisture content at field capacity as well as increases in bulk density and moisture content at permanent wilting point under severe moisture deficit (35% FC watering) was consistent with the findings of Korenkova and Uric [33] who obtained increases in soil bulk density and decrease in porosity of soil under suboptimal watering. Such deformation of soil due to deficit irrigation might account for reduced growth and vigour observed among the studied urban forest trees species (UFTS).

The repetitive wetting and drying of soil under suboptimal watering (35% FC) might restrict water and air transport and facilitate formation of preferential flow path due to deformation of soil matrix and changing configuration of structural pores [34]. Further, soil structure and water holding capacity are at best in optimal watering condition while mineral nutrients are optimally available for plant uptake [35]. Thus, suboptimal watering treatments imposed mild and sever moisture deficit stress in addition to reduction in soil organic matter and other nutrient elements and poor structure enhanced reduction in water holding capacity. These results agree with the findings of Sardans et al. [36] and Badiane et al. [37] that suboptimal soil moisture reduced the quantity and mineralization of soil organic matter. Decrease in the rhizosphere pH and electrical conductivity indicates increasing acidity as the soil dries out which probably resulted from restricted movement of dissolved cations, declined organic matter solubility and repressed microbial activities. The lack of significant difference in available macro nutrients among treatments suggests high soil moisture deficits as obtained under 35% FC watering did not significantly reduce dissolution of soil essential cations which will be beneficial for plant growth [38].

Table 2a. Responses of growth attributes of UFTS seedlings

Species	Shoot height cm	Number of leaves	Stem girth cm	Leaf area cm^2	Total leaf area cm^2	Root length cm	Plant fresh weight g	Root Dry weight g	Plant dry weight g	Root: shoot ratio	Dickson quality index g
B. monandra	47.791b	8.532b	2.517c	68.353bc	823.223bc	30.967bc	53.141b	6.508c	21.340c	0.404b	0.992c
D. regia	57.123a	6.817bc	4.181a	164.283a	1318.955b	36.630a	103.968a	12.943a	44.610a	0.492a	2.687a
D. lutescence	21.918c	4.952c	2.513c	47.096c	295.232c	25.766d	18.817c	1.441d	4.561d	0.438b	0.410d
T. catappa	53.890ab	25.627a	3.732b	88.717b	3085.371a	35.412ab	95.977a	10.210b	37.715b	0.405b	2.238b
V. merrillii	28.640c	6.227bc	2.479c	61.136c	521.243c	30.211cd	26.153c	2.002d	6.732d	0.420b	0.476d
LSD (P<.05)	9.332	3.580	0.449	27.581	797.712	5.200	26.988	2.733	6.896	0.053	0.449

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance

Table 2b. Responses of Physiological Attributes of UFTS Seedlings

Species	Relative water content	Plant growth rate	Relative growth rate	Leaf area ratio	Specific leaf area	Net assimilation rate × 10^{-4}	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a/b ratio	Carotenoids	Carotenoids/ Chlorophyll ratio	Water applied	Plant water use	Water use efficiency
Units	%	g/day	g/day	cm2/g	cm2/g	g/day	µM mg^{-1}FW				µM mg^{-1}FW		L	L/day	L/g
B. monandra	59.532d	0.158c	0.013b	2.934c	178.498b	19.750ab	7.909d	5.882c	13.791d	1.396bc	2.943c	0.280a	6.961d	0.783d	4.152b
D. regia	65.792c	0.459a	0.021a	3.924b	2457.211a	16.540b	17.830bc	16.610a	32.685b	1.517ab	3.988b	0.129b	8.009a	2.065a	5.555a
D. lutescence	73.036b	0.047d	0.014b	8.550a	95.248b	6.507c	16.276c	10.844b	27.121c	1.744a	6.581a	0.252a	5.036a	1.353c	0.846c
T. catappa	76.509a	0.281b	0.010c	2.137d	200.544b	21.628a	18.982ab	16.430a	35.412a	1.160c	3.524b	0.116b	7.961a	1.528bc	4.659b
V. merrillii	78.125a	0.057d	0.013b	8.301a	134.730b	6.154c	20.260a	17.407a	37.667a	1.254bc	2.872c	0.092b	4.714b	1.811ab	1.540c
LSD (P<.05)	3.472	0.100	0.007	0.797	2256.667	10.030	2.429	4.962	2.727	0.348	0.581	0.123	1.000	0.282	0.896

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance

Table 2c. Biomolecules accumulation in the examined UFTS seedlings

Species	Superoxide Dismutase	Guaiacol Peroxidase × 10^{-3}	Catalase × 10^{-2}	Total flavonoids	Total phenolic acids	Ascorbic acid	Total soluble sugar	Malondialdehyde (MDA) × 10^{-3}	Crude protein	Proline
Units	Units min^{-1}mg^{-1}FW	µM min^{-1} mg^{-1}FW	µM min^{-1} mg^{-1}FW	mgg^{-1} QE	mgg^{-1} GAE	mM g^{-1}FW	mg/100mg	nM ml^{-1}	%	µM g^{-1}FW
B. monandra	10.161a	1.090a	3.520c	80.120c	423.135d	1.722c	11.670e	8.766a	13.982c	7.395c
D. regia	10.830a	0.728c	5.436a	91.302b	504.569bc	2.291b	21.991a	5.303b	26.553a	14.623ab
D. lutescens	7.549c	0.614d	3.285c	93.550b	450.845cd	2.229b	13.499d	5.630b	3.607e	12.692b
T. catappa	8.498bc	0.765bc	4.524b	93.717b	556.263b	2.551b	16.204b	5.013b	24.842b	16.216a
V. merrillii	8.958b	0.839b	4.600b	108.195a	631.550a	3.234a	14.905c	3.888c	11.993d	14.782ab
LSD (P<.05)	1.204	0.074	0.836	11.182	51.694	0.507	1.406	1.125	1.711	1.931

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance.

Table 3a. Effects of biochar amendment on morphological attributes of five UFTS seedlings

Treatments	Shoot Height	Number of Leaves	Stem Girth	Total Leaf Area	Plant Fresh Weight	Plant Dry Weight	Root: Shoot Ratio	Dickson Quality Index
Units	cm		cm	cm^2	g	g		g
Biochar Treatment	46.368a	11.825a	3.336a	1546.939a	64.699a	24.843a	0.424a	1.427a
No Biochar	37.377b	9.037b	2.833b	870.671b	54.524b	21.140b	0.439a	1.294a
HSD (P<.05)	0.000	0.000	0.000	0.000	0.016	0.038	0.082	0.158

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance.

Table 3b. Response of physiological characters of examined UFTS Seedlings to Biochar amendment

Treatments	Relative water content	Plant growth rate	Relative growth rate	Leaf area ratio	Specific leaf area	Net assimilation rate	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a/b ratio	Carotenoids	Carotenoids/ Chlorophyll ratio	Water applied	Plant water use	Water use efficiency
Biochar Treatment	71.954a	0.217a	1.504a	5.471a	650.576a	1.420a	17.028a	14.594a	30.920a	1.421a	3.595b	0.147b	6.284a	1.630a	2.497b
No Biochar	69.244b	0.183a	1.346a	4.867a	575.917a	1.403a	15.475b	12.275b	27.750a	1.407a	4.369a	0.201a	6.788a	1.382b	3.041a
HSD (P<.05)	0.001	0.053	0.156	0.033	0.350	0.894	0.003	0.000	0.000	0.868	0.000	0.000	0.060	0.001	0.011

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance.

Table 3c. Biochemical accumulation in UFTS Seedlings under Biochar-soil amendment

Treatments	Superoxide Dismutase	Guaiacol Peroxidase × 10-4	Catalase × 10-2	Total Flavonoids	Total Phenolic Acids	Ascorbic Acid	Total Soluble Sugar	Malondialdehyde (MDA) × 10-3	Crude Protein	Proline
Biochar Treated	9.566a	8.706a	4.964a	84.376b	498.018b	2.146b	14.715b	5.247b	17.045a	11.247b
No Biochar	8.832b	7.439b	3.581a	102.377a	528.527a	2.665a	16.607a	6.191a	15.346b	15.037a
HSD (P<.05)	0.004	0.000	0.000	0.000	0.033	0.000	0.000	0.001	0.024	0.000

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance

Table 4a. Effects of watering regime on some growth attributes of UFTS seedlings

Treatments	Shoot height	Number of leaves	Stem girth	Leaf area	Total leaf area	Root length	Plant fresh weight	Plant dry weight	Root: shoot ratio	Water applied	Plant water use
Units	cm		cm	cm^2	cm^2	cm	g	g			
35% FC	33.056b	7.797a	2.547b	61.731b	571.288b	29.776a	51.004a	19.948a	0.447a	5.395c	1.116b
60% FC	38.241ab	9.106a	2.940a	77.023ab	905.969ab	31.554a	53.553a	21.071a	0.438a	6.697b	1.443a
80% FC	40.836a	10.207a	3.013a	86.984a	1134.755a	32.134a	59.015a	22.401a	0.433a	7.904a	1.599a
LSD (P<.05)	7.780	2.410	0.393	25.254	334.681	2.358	2.549	1.123	.006	1.207	0.328

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance. 80% FC watering denotes optimal watering or control, 60% FC denotes mild watering and 35% FC denotes severe watering treatment

Table 4b. Effects of Watering Regime on Physiological Attributes of UFTS Seedlings

Treatments	Relative water content	Plant growth rate	Relative growth rate	Leaf area ratio	Specific Leaf Area	Net assimilation rate	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a/b ratio	Carotenoids	Cararotenoids/ Chlorophyll	Water Use Efficiency
Units	%	g/day	g/day $(\times 10^{-2})$	g/cm^2	g/cm^2	g/day $(\times 10^{-3})$							
35% FC	68.132a	0.168a	1.305a	4.603a	456.847a	1.447a	14.423b	11.029b	25.452b	1.505a	4.582a	0.225a	3.523a
60% FC	69.139a	0.181a	1.342a	4.889a	603.565a	1.383a	15.558ab	12.339ab	27.898ab	1.381a	4.428a	0.201a	3.076a
80% FC	70.460a	0.201a	1.393a	5.110a	667.338a	1.378a	16.442a	13.458a	29.901a	1.337a	4.097a	0.178a	2.580a
LSD (P<.05)	1.007	0.013	0.037	0.507	146.718	0.005	2.019	1.311	2.446	0.044	0.485	0.047	0.943

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance. 80% FC watering denotes optimal watering or control, 60% FC denotes mild watering and 35% FC denotes severe watering treatment.

Table 4c. Biochemical constituents of UFTS as affected by watering regimes

Treatments	Superoxide Dismutase	Guaiacol Peroxidase	Catalase $(\times 10^{-2})$	Total Flavonoids	Total Phenolic Acids	Ascorbic Acids	Total Soluble Sugars	Malondialdehyde (MDA)	Crude Protein	Proline
Units	Units min^{-1}mg^{-1} FW	µM min^{-1} mg^{-1}FW $(\times 10^{-4})$	µM min^{-1} mg^{-1}FW	mgg^{-1} QE	mgg^{-1} GAE	mM g^{-1}FW	mg/100mg	nM ml^{-1} $(\times 10^{-3})$	%	µM g^{-1}FW
35% FC	8.010b	6.236b	1.847c	113.131a	603.931a	2.914a	16.446a	7.761a	13.454b	18.667a
60% FC	9.005ab	7.762a	4.001b	100.175a	543.112b	2.567a	14.062b	5.833b	15.570b	13.800b
80% FC	9.481a	8.320a	4.896a	93.824b	438.538c	2.514a	13.421b	4.985b	17.015a	12.644b
LSD (P<.05)	0.995	1.534	0.009	6.351	60.819	0.052	2.384	1.928	1.445	4.867

Values along the column bearing same letters are not significantly different at α = 0.05 level of significance. 80% FC watering denotes optimal watering or control, 60% FC denotes mild watering and 35% FC denotes severe watering treatment

4.2 Effects of Biochar Amendment on Soil Properties

Decreased bulk density due to biochar amendment of the variously watered soil supports published reports on biochar enhancement of soil properties [39,40]. The creation of micro- and meso-pores from the retained cell wall of feedstock during pyrolysis of feedstocks decreased bulk density under biochar amendment of mineral soils [41] due to increased pore volume of biochar-soil mix [42]. The increasing bulk density observed with decreasing watering levels could be related to decreased soil pH and repressed microbial activities which probably lower the organic matter solubility via alteration to charge density of humic compounds [43].

The meso and micro-pores of biochar might provide a large surface area for intermolecular attraction forces between biochar and water molecules, thus, allowed biochar to act like a sponge, soaking up water and retain more moisture [44]. Thus, our results confirm reports of biochar amendment as viable water-saving strategy for improving soil properties and plant growth Biochar application had reportedly increased soil water holding capacity.at field capacity [45] and under drought conditions [46]. The improved soil water status such as the high soil moisture at field capacity, plant available water content (PAWC) and total available water (TAWC)] due to biochar amendment would enhance plant water uptake (moisture depletion) to lower water content before wilting is attained. Such positive effects of biochar at suboptimal watering levels could be associated with hydroxyl and carboxylic groups on biochars surface connecting soil micro-aggregates (adsorbing soil particles and clays) [47] leading to formation of additional macro-aggregates from micro-aggregates through soil particle rearrangement [41]. Uzoma et al. [48] and Basso et al. [18] also reported increased TAWC and WHC of sandy loam soil with biochar amendment. Nguyen et al. [49] linked the increased level of TAWC due to switchgrass biochar amendment to increased soil moisture availability.

Increased soil pH of biochar-amended soil could be related to biochar capacity to decrease the soil exchangeable Al^{3+} content through binding Al^{3+} ion (and soluble Fe) with oxygenated functional groups on its surface, thus, increased the abundance of soil exchangeable base cations and base saturation [50]. Alternatively,

ash accretion might have resulted from the high ash content (23.9%) of the applied biochar causing neutralization of the acidic soil. High soil organic matter could be ascribed to high porosity of biochar which possibly increased the activity of carbon decomposers in agreement with the findings of Wang et al. [51].

The decomposition of soil organic carbon (SOC) is highly sensitive to soil moisture level [52]. Thus, augmentation of SOC and nitrogen contents could be well related to additional water molecules gradually released from micro and meso-pores of the applied biochar as the soil dries out. Further, high porosity and microbial attractiveness of biochar can enhance soil biological processes such as mineralization, nitrification and other mineral-solubilization activities [53]. Coupled with characteristic high temperature and aeration prevalent during this experiment, the slightly acidic rhizosphere pH and moderate moisture contents provided by biochar are factors in favour of rapid microbial decomposition of native SOC [54]. Liming effects of the applied biochar might aid microbial population and activities which increased N mineralization and nitrification in agreement with the findings of Novak et al. [55].

Biochar can reduce denitrification via its affinity to retain and facilitate uptake of NH4+ by plants, leading to reduced nitrogen availability for denitrification bacteria [56]. The high specific surface area of biochar mainly for anionic adsorption can improve rhizosphere N by reducing nitrate leaching or absorb and store nitrate ions in the soil [53]. The UFTS seedling did not show any symptoms of nitrogen immobilization in biochar-treated soil because C: N of 26:1 (optimal watering) and 28:1 (in severe watering) is apparently ideal for microbial decomposition of organic matter. Cox et al. [57] stated that biochar carbon is stable and not available for decomposition, therefore, the ratio 25-30 parts carbon to one part nitrogen (C:N ratio) prevents nitrogen drawdown (temporary loss of available nitrogen) and therefore may not result to nitrogen immobilization in biochar amended soil. Increased soil pH due to biochar amendment has implications on mineralization and availability of Ca, Mg and K [58]. Thus, suitable pH in this study might facilitate direct replenishment of the soil with soluble labile cations (K^+, Ca^{2+} and Mg^{2+}) which agrees with the findings of Page-Dumroese et al. [59] that biochar produced from wood waste materials (sawdust) contains high levels of soluble K, P

and Ca which upon incorporation into soil could be released to promotes plant growth. Further, biochar might facilitate absolute dissolution of cations in the native soil due to oxidation of carboxylate and other ionizable functional groups on its surface [60]. Augmentation of plant available Ca^{2+} and Mg^{2+} could be related to liming potential of biochar while provision of microhabitat for phosphate-solubilizing bacteria genera and microbes involved in N and S transformation may account for increased level of available PO_4^{3+} in biochar amended soils [61].

4.3 Response of Growth Traits and Biochemical Constituents of UFTS Seedlings

Plant tolerance to drought stress is directly related to the genetic composition of the species, degree of stress, the interactions among stress factors, and their developmental stages [62]). In this study, *Delonix regia* was superiorly taller with thicker stem, had larger leaf, deeper root system, greater fresh and dry weights, root: shoot ratio, seedling vigour, growth rate and specific leaf area which reflects better performance in suboptimal watering condition than other evaluated UFTS. This could be related to the effective activities of superoxide dismutase and catalase as well as high soluble sugar content in *D. regia* seedlings compared with other UFTS. The relatively high root: shoot ratio and large canopy-accompanied high transpiration rate of *D. regia* demonstrated its high capacity for use as street, parks, avenues and plantation trees and for wasteland recovery. *T. catappa* showed better leaf traits adaptation by having high number of leaves and total leaf area. Coupled with this, high water use efficiency and net assimilation rate bestowed better commercial (aesthetic) value on this species than other investigated UFTS. Further, high proline in *T. catappa* leaf might account for higher leaf turgidity and chlorophyll accumulation but lower malondialdehyde (MDA) lipid peroxidation compared with *D. regia, B. monandra* and *D. lutescens*. Thus, *T. catappa* could be quite an excellent representative of street and avenue trees and could make a park view more spectacular in dry urban settlement.

B. monandra exhibited the least root: shoot ratio, leaf turgidity, chlorophyll, proline, ascorbic acid, flavonoids, phenolic acid and soluble sugar contents which culminated into having highest MDA lipid peroxidation. Further, high carotenoids: chlorophyll ratio could mean striving for shielding chlorophyll system from oxidative damage. Maintenance of appropriate water status under suboptimal watering condition is achieved by stomata regulation [63] and accumulation of compatible solutes [64]. Thus, accumulation of high chlorophyll, ascorbic acid, phenolic acids and flavonoids contents as well as relatively high activities of catalase and guaiacol peroxidase may account for high leaf turgidity and least malondialdehyde lipid peroxidation in young *V. merrillii*. This species required the least irrigation volume for best performance in the nursery, thus, may be an excellent candidate for use as street, avenue and park tree and horticultural garden especially under future dry climate in cities.

4.4 Biochar Effects on Growth Traits and Biochemical Constituents of UFTS Seedlings

Biochar increased morphological attributes such as shoot height, number of leaves, stem girth, total leaf area, whole plant fresh and dry weights of UFTS by augmenting photosynthetic pigments, osmolytes accumulation and through up-regulating enzymatic antioxidants activities which led to low MDA lipid peroxidation in the cities-adapting UFTS. In this study, addition of biochar improved soil physical and chemical properties which might facilitate enhanced growth attributes under suboptimal watering conditions. Thus, improved growth traits of UFTS in suboptimal watering condition could be ascribed to improved soil structure, fertility and water status caused by biochar amendment. Chan et al. [65] attributed biochar enhancement of plants growth to increased nutrient availability and improved soil properties via reduced bulk density. Additionally, constant maintenance of high moisture content in the rhizosphere alleviates stress and enables UFTS to grow better, thus, suggests biochar as an excellent amendment for improving WUE and drought tolerance of UFTS. Overall, application of biochar at suboptimal watering might be a viable technique for conserving water resources in urban areas while enhancing productivity and aesthetic quality of UFTS simultaneously.

The high leaf turgidity in biochar-grown UFTS could be traced to overall augmentation of the soil available K^+ resulting from K^+ rich biochar applied that promoted K^+ absorption. This observation agrees with previous studies that biochar application increased availability and uptake of nutrients in water-stressed plants:

tomato [66], maize [+67] and soybean [10] leading ultimately to increased leaf turgidity. Similarly, biochar enhancement of chlorophyll accumulation in suboptimal watering condition could be traced to either promotion of chlorophyll biosynthesis via improving the soil nutritional status; in particular, the available nitrogen (N) and magnesium (Mg) or inhibition of chlorophyllase activity. The improved availability and uptake of Mg and N which are essential structural components of chlorophyll might augment chlorophyll accumulation in the biochar-treated UFTS. Lehmann et al. [68] stressed that in stress condition, biochar addition may not only improve the nutrients availability but promote vegetative growth by improving the photosynthetic chlorophyll content. Biochar maintaining constant high water level in the rhizosphere boosted total photosynthetic performance index of biochar-grown UFTS either by increasing soil-plant-water relationships, electron transport rate of PSII and reducing stomatal conductance as observed for maize [67] or reducing oxidative damage to the photosynthetic apparatus by regulating the activity of protective enzymes [69].

Biochar enhancement of the activities of superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPOx) seemingly protected UFTS from injurious effect of reactive oxygen species (ROS) by creating ionic homeostasis at the cellular level leading to low level of malondialdehyde (MDA) lipid peroxidation in biochar-treated UFTS. Such high activities of antioxidant enzymes accompanied by reduced lipid peroxidation suggests alleviation of oxidative damage to membrane lipids as a viable drought stress mitigation strategy of biochar and as such demonstrates the beneficial roles of biochar as plausible nursery amendments for UFTS production, survival and establishment on dry sites. Alternatively, biochar-induced alleviation of oxidative stress in UFTS may be associated with improved physical, chemical and biological characteristics of soil which facilitated availability and uptake of mineral nutrients and water, augmented water use efficiency and upregulated the conversion of $O_2^{\cdot-}$ to H_2O_2 and O_2 by superoxide dismutase and subsequent scavenge of toxic H_2O_2 to H_2O and O_2 by catalase and guaiacol peroxidase. Biochar enhancing activities of SOD, GPOx and CAT have been previously reported [70]. Similarly, reduced level of osmolytes and osmoprotectants was an attestation to reduced oxidative stress which promoted growth attributes in biochar

grown UFTS. At suboptimal water level, biochar enhanced soil porosity, pH and availability of water and mineral nutrients, accumulation of proline, soluble sugar, ascorbic acid and phenolic acids. Biochar augmentation of osmolytes and osmoprotectants is widely reported [71,72].

This result agrees with previous studies that biochar addition at suboptimal water level strengthened plant defense mechanisms by up-regulating pathways and genes associated with plant defense, thus, increased drought tolerance [73] and water-use efficiency of [74] of ready-to-transplant species of plants.

4.5 Watering Regime Effects on Growth and Biochemical Constituents of UFTS Seedlings

Optimal (80% FC) watered UFTS exhibited the best growth adaptations than those in suboptimal (60% and 35% FC) watering regimes which shows that water should be adequately and continuously made available for production of vigorous UFTS seedlings and also agrees with the findings on *Brachystegia eurycoma* seedlings [75]. This observation could be ascribed to maximization of light interception through investing a considerable fraction of photo-assimilates under optimal watering condition. Nevertheless, maintenance of acceptable growth rate in severe watering condition reflects high adaptation of UFTS to suboptimal watering in this region.

High proline and soluble sugar accumulation in suboptimal watered UFTS seedlings may be attributed to proline effect on turgor potential maintenance and consistent with reports for black poplars [76], mulberry [77], eucalyptus [78], oaks [79] and Conocarpus, Salix and Acacia [80] plants.

Under moisture deficit condition, phenolic compounds acts as antioxidants [81]; neutralizing free radicals (ROS) by quenching singlet and triplet oxygen and/or decomposing peroxides developed in the chloroplasts [82]. Such responses may result in maintenance of photosynthetic apparatus and membrane cell integrity. The increased levels of phenolic compounds in UFTS seedlings may serve in activating defense mechanisms via up-regulating phenolics-synthesizing enzymes, such as phenylalanine ammonia-lyase [83] which supports the findings on *Salix* and *Acacia* [84], *Portulaca oleracea* [85], *Eucalyptus globulus*

[86], *Syzygium cumini* [87] and canola [88] in suboptimal watering condition.

Increased activities of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOx) at suboptimal irrigation confirms the role of antioxidant enzymes system in protection of UFTS cellular organelles from toxicity effects of reactive oxygen species (ROS). It appears that the UFTS seedlings initiates defense mechanisms through activation of SOD which functions as both precursor for highly reactive oxygen derivatives - peroxynitrite and hydroxyl radical [89] and as ROS scavenger through dismutation of 0_2 to H_2O_2 in suboptimal watering condition [11]. Thus, up-regulation of the activity of CAT and GPOx by UFTS decreased cellular H_2O_2 and therefore improve tolerance of these species to suboptimal watering. Similar enhancement of the activities of SOD, POD and CAT was reported for *Morus alba* and *Conocarpus erectus* [91], *Acacia modesta* and *Salix tetrasperma* [80], peach [91], grapes [92] and olive plants [93]. Chlorophyll pigments loss in UFTS under suboptimal watering treatment supports the possible involvement of antioxidants system in preserving the PSII functional integrity in water stressed plants. McKinnon and Mitchell [94] opined that chlorophyll loss is a regulatory mechanism to reduce light harvesting and enhance photo protection in plants under soil moisture deficit. Oxidative burst injures the cellular organelles, protein structure, causes nucleic acid fragmentation and impairs other physiological processes [95] leading to increased malondialdehyde (MDA) lipid peroxidation. In plants, the damage of membrane lipids increased MDA content of UFTS [96]. In this study, suboptimal watering induced ROS generation in UFTS seedlings. The high peroxidation of membrane lipids may pose threat to survival and establishment of UFTS under suboptimal watering condition. These results corroborate the reports on *Jathropha curcas* [97], *Populus kangdingensis* and *P. cathayana* [98], pistachio (Khoyerdi et al. 2016).

5. CONCLUSIONS

Differential watering affected soil physical and chemical properties and growth performance of seedlings of UFTS evaluated. The responses of soil properties and physiological traits of urban forest trees species (UFTS) to watering regimes was species specific. Deficit irrigation increased bulk and particle densities, reduced soil moisture content at field capacity but increased moisture at permanent wilting point. Total nitrogen, available K^+, Ca^{2+} and Mg^{2+} reduced slightly under mild (60 %FC) and severe (35 %FC) moisture stress conditions compared with watering at 80 % FC. Addition of biochar to the variously watered soil considerably reduced bulk density and remarkably increased porosity and field capacity moisture. Biochar amendment also increased soil pH, soil organic matter and volatile organic matter contents, available K^+ and Ca^{2+}, extractable Mg^{2+} and dissolved phosphate (PO_4^{3+}) remarkably relative to watering at 80 % FC.

Seedling growth attributes significantly reduced under deficit water application (60 and 35 % FC) relative to 80 % FC. Watering regime had substantial effects on accumulated osmolytes, osmoprotectants and enzymatic activities of UFTS seedlings. Deficit watering (60 and 35 % FC) induced remarkable accumulation of osmolytes and osmoprotectants and enzymatic activities. Watering at 80% field capacity produced optimal growth, development and vigour of UFTS seedlings which may enhance field establishment and reduce mortality. Biochar amendment of the variously watered soil enhanced accumulation of osmoprotectants and up-regulated enzymatic activities of superoxide dismutase, guaiacol peroxidase and catalase in UFTS seedlings. Biochar amendment may serve as effective strategy for improving soil properties and performance of UFTS seedlings thus mitigating the adverse effects of suboptimal watering on soil and plant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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