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Effects of Water Application Rates and Sawdust Biochar on the Physicochemical Properties of Soil and Performance of Five Tree Species Used in Urban Landscaping in Ondo, Nigeria

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Effects of Water Application Rates and Sawdust Biochar on the Physicochemical Properties of Soil and Performance of Five Tree Species Used in Urban Landscaping in Ondo, Nigeria

This study assessed the response to irrigation rate and the minimum irrigation rate for optimum growth of seedlings of five tropical tree species used in urban landscaping in Ondo, Nigeria. The study also evaluated the effects of irrigation rate and sawdust biochar on growth attributes and biochemical constituents of the seedlings and the changes in the physical and chemical properties of the soil on which the tree seedlings were grown. Seedlings of five tree species, Bauhinia monandra, Delonix regia, Terminalia catappa, Dypsis lutescens, and Veitchia merrillii, were subjected to six treatments as follows; The first three treatments comprised of seedlings grown in soil without biochar application and subjected to 80%, 60%, and 35% FC irrigation rates while the last three treatments were seedlings grown in the soil-biochar mixture and subjected to 80%, 60%, and 35% FC irrigation rates. Seedlings grown in soil irrigated at 80% FC served as control. Relative to 80% FC treatment, soil bulk density increased, moisture content decreased significantly, and plant available K, Ca, and Mg and percentage N decreased transiently in the soil subjected to 60% and 35% FC irrigation rates. There were no significant differences between the measured growth attributes and malondialdehyde content of the seedlings irrigated at 80% FC and those irrigated at 60% and 35% FC. In addition, seedlings in the 35% and 60% FC treatments deployed non-enzymatic and enzymatic antioxidant machinery to overwhelm the oxidative burst in their chloroplasts. Conclusively, the minimum irrigation rate for optimum growth of the tree seedlings was 35% FC. The practice of a 60% and 35% FC irrigation rate for tree production in the nursery was a viable water-saving strategy, and a 35% FC watering rate was beneficial for enhancing the tolerance of the tested trees. Irrespective of irrigation rate, biochar application improved soil's physical and chemical properties, moisture content, and plant-available macronutrients. Biochar amendment also produced the most vigorous seedlings at an 80% FC irrigation rate, enhanced the measured attributes of seedlings irrigated at 60% FC, alleviated the adverse effects, and strengthened the tolerance of tree seedlings in the 35% FC watering rate.

Keywords

irrigation rate, water-saving strategy, enzymatic antioxidant activity, nursery operators, tropical tree, water stress

INTRODUCTION

Trees constitute an essential element of the cityscape and serve various environmental and socioeconomic benefits (Arnold 1993). The graceful shape of trees provides an architectural transition between human size and the scale of buildings and streets (Jacobs 1995; Bell et al. 2005). In the past, food crops were the main reason for planting trees in towns and cities. However, trees can thermostatically decrease heat intensity, the energy needed for cooling buildings in the cities, and act as noise filters (Akbari et al. 2001; Maco and McPherson 2003; King and Davis 2007). They also act as air purifiers, capturing air pollutants such as particulate matter, carbon dioxide, and ozone originating from transport and industrial activities (McPherson et al. 1997). Many inhabitants of Nigerian cities cultivate various tree species around their homes and workplaces because they love to live and work in a green environment (Arabomen et al. 2016). Many workplaces in urban areas like; schools, event and club centers, hotels, banks, castles, markets, and fuel stations are decorated with attractive trees for recreational purposes (Tyrväinen et al. 2005). Nigerian cities are, therefore, densely populated with exotic trees compared with indigenous species. Akachukwu (1998) noted that natural forests are fast declining in tropical urban areas due to urbanization, industrialization, ever-increasing population, and the expanse of land required for road construction. It is, therefore, necessary to develop plant-based urban green infrastructure for health and energy improvement in our cities.

Tree seedlings are often grown during the dry season for out-planting immediately after the onset of the following rainy season. However, high rates of evapotranspiration that dry up most rivers, streams, shallow wells, and boreholes during the dry season often worsen the plight of poor resource-endowed nursery operators. High industrial and domestic demand for municipal water in southwest Nigeria also complicates the hardships faced by private nursery operators by making water unavailable for irrigating horticultural tree seedlings. Thus, water availability for nursery practice has become a challenge to urban greening in the region. Previous workers observed that optimal watering at the nursery stage improved seedlings' quality and allowed the rapid establishment of transplants on the field (Gbadamosi 2014; Daba and Tadese 2017; Ogidan et al. 2018), whereas suboptimal watering resulted in poor quality planting stock, and decreased the survival rate and lifespan of the out-planted trees (Oboh and Igharo 2017). Water stress can decrease the mineralization and mobility of nutrients and make nutrients unavailable for root uptake (Mannan et al. 2016). It can also reduce leaf water potential, stomatal conductance, transpiration, and net photosynthetic rate in plants (Farooq et al. 2009). Water stress can trigger the over-excitation of photosynthetic pigments in the antennae and produce excessive active oxygen species (ROS) that destroy chloroplast membrane structure (Mittler 2002; Munné-Bosch et al. 2003). Although nursery growers in southwest Nigeria are exploring various irrigation techniques like; scheduled irrigation and deficit irrigation, the insufficient scientific data on optimal water demands and drought tolerance of tropical tree species will aid nursery practitioners in making informed decisions on their operations.

A class of commercial nurseries has started procuring efficient irrigation facilities and adopting the planting of water-use-efficient tree seedlings to expand urban greening in the region. However, some species of plants are well equipped with effective antioxidant defense systems that suppress the water stress effect in sub-optimal irrigation conditions. The first system (enzymatic antioxidant defense system) involves the activation of enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (GPOx), and catalase (CAT), whose primary functions are

to scavenge water stress-induced ROS and reduce phytotoxic H2O2 to a non-toxic water molecule. The non-enzymatic antioxidant defense mechanism involves the accumulation of biomolecules such as proline, ascorbic acid (ASC), carotenoids (Car), phenolic acids (TPC), and flavonoid (TFC), which either substitute water molecules during tissue dehydration (osmolytes) or reduce phytotoxic H2O2 to non-toxic substance. However, studies that evaluated the responses and minimum irrigation demand of landscape tropical tree species to the watering regime in the nursery are very scanty. It is, therefore, necessary to evaluate the performance and tolerance of tropical tree species commonly used for urban landscaping in water stress conditions to establish suitable tree species for greening the cities in southwest Nigeria.

Biochar is a fine-grained, porous carbon substance produced by heating biomass-derived feedstock in low-oxygen conditions and intentionally added to soil to improve soil health and sequester carbon (Lehmann 2007). Because of its unique characteristics like; high porosity, cation exchange capacity, sorptive capacity, ease of production, etc., biochar technology appears as a promising strategy for conserving water resources input while improving soil and tree stock quality in the nursery. In other words, the inclusion of biochar in soil for growing landscape tree seedlings appears as a long-term, all-in-one package solution to the challenges of scarcity of irrigation water and urban greening in tropical areas. Previous workers suggested that if incorporated at an appropriate dose, biochar can increase the moisture content, water, and nutrient retention capacity of the soil (Sarong and Orge 2015; Sohi et al. 2010), mineralization and retention of soil nitrogen (Korenkova and Uric 2012), root uptake of water and nutrient, nitrogen use efficiency, growth, and yield of tree crops (Eyles et al. 2015; Anjum et al. 2011). Biochar technology can aid the conservation of scarce municipal water resources by decreasing irrigation frequency and rate (Basso et al. 2013) and ease root penetration and nutrient mobility by reducing soil bulk density.

This study assessed the minimum irrigation rate for growing seedlings of five tropical tree species used in urban landscaping in southwest Nigeria. The study further evaluated the single and combined effects of irrigation rate and sawdust biochar on growth attributes and biochemical constituents of the five tree species at the seedling stage. To holistically understand biochar's contribution to alleviating water stress at the seedling stage, this study analyzed the changes in the physical and chemical properties of soil used in growing the investigated tree species. The hypothesis was that biochar improves the soil's physical and chemical properties and subsequently alleviates the effect of water stress on growth attributes and seedling vigor of the five tree species.

MATERIALS AND METHODS

Study site, plant, and soil materials

This experiment spanned between December 1, 2020, and June 30, 2021, and was conducted in the Screen House of the Department of Biological Sciences, Wesley University, Ondo, Nigeria. The averages of the upper and lower temperatures of the Screen House were 25.1 and 18.2 °C, and the average relative humidity was 62%. Soils collected at the botanical garden, Wesley University, and sawdust collected at a sawmill Ife garage, Ondo, Nigeria, were utilized in the experiment. The sawdust comprised about 45, 40, and 15% of the dust from *Gmelina arborea*, *Khaya senegalensis*, and other tree species. After air-drying sawdust until the moisture level was

<10%, it was pyrolyzed in a local reactor at 500 °C for 5 hours of residence time to produce sawdust biochar. Before planting, the garden soil and sawdust biochar were analyzed, and the results of physical and chemical properties of soil and biochar are shown in Table 1.

Experimental setup

Approximately 1950 plastic pots (Upper × Lower diameter × Height = 26 × 20 × 30 cm) were perforated at the bottom and separated into two equal groups. About 975 plastic pots in the first group were filled with 10 kg garden soil (no biochar application), and the remaining 975 pots in the second group were filled with a mixture of 9.6 kg garden soil and 0.4 kg sawdust biochar (given an equivalence of 4 % w/w or 52 t ha-1 soil-biochar mixture). Soil and biochar were mixed thoroughly with a hand trowel. Five tree species commonly used in urban landscaping in Ondo, Nigeria, were investigated. *Bauhinia monandra* Kurz, *Delonix regia* (Bojer ex Hook) Raffin, and *Terminalia catappa* L. are the prevalent semi-deciduous species, whereas *Veitchia merrillii* (Becc.) H. E. Moore, and *Dypsis lutescens* (H. Wendl.) Beentze and J. Dransf are the most prevalent palm tree species used for decoration in Ondo, Nigeria. Both *V. merrillii* and *D. lutescens* are slow-growing palm species and appeared too young to be subjected to water stress until they were six months old.

The experiment commenced when six-month-old seedlings of *V. merrillii* and *D. lutescens*, and two-month-old seedlings of *B. monandra, D. regia,* and *T. catappa,* were transplanted at one seedling per pot. Approximately 390 seedlings of each species (totaling 1950 seedlings for the five tree species) were randomly allotted to six treatments as follows; Seedlings grown on garden soil and subjected to 80%, 60%, and 35% field capacity (FC) irrigation rates served as control, mild and severe treatments respectively and were coded as T1, T2, and T3 respectively while seedlings grown on the soil-biochar mixture and subjected to 80%, 60%, and 35% FC irrigation rates served as biochar with adequate, mild and severe irrigation respectively and were coded as T4, T5, and T6 respectively. There were 65 seedlings per treatment. Field capacity (FC) was measured by weighing each pot gravimetrically at 3-day intervals to determine water loss through evapotranspiration. Therefore, at 3-day intervals, seedlings in the T1 and T4 regimes were irrigated with distilled water equivalent to 80% evapotranspiration water loss, seedlings in the T2 and T5 regimes were irrigated with distilled water equal to 60% evapotranspiration water loss, and seedlings in the T3 and T6 regimes were irrigated with distilled water equivalent to 35% evapotranspiration water loss. The significance of T2 and T3 treatments was to assess the response of the five tree species to watering rate and establish the minimum irrigation demand of each tree species in the nursery, and T4, T5, and T6 treatments aimed at evaluating biochar's potential to alleviate water stress on growth attributes and seedlings vigor of each species. All seedlings were arranged randomly in the screen house.

Table 1: Physical and chemical properties and elemental composition of the top garden soil and sawdust biochar

Physical and chemical properties	Soil	Biochar
Sand (%)	66.62	na
Clay (%)	15.44	na
Silt (%)	17.94	na
Total porosity (%)	30.45	85.87
Bulk density (g cm^{-3})	1.4	1.14
Textural class	Sandy loam	na
Moisture content at field capacity (%)	26.57	2.89
Moisture content at permanent wilting point (%)	8.47	nd
Total available water content (%)	35.04	2.89
Water holding capacity (%)	26.57	4.02
$pH_{(H2O)}$	5.81	9.72
Electrical conductivity (dS m^{-1})	0.63	1.05
Soil organic matter (%)	3.12	nd
Total organic carbon (%)	1.804	66.3
Total nitrogen content (%)	0.66	1.02
Carbon/Nitrogen ratio	11:01	65:01
Phosphorus (mg kg^{-1})	8.1	30.697
Potassium (mg kg^{-1})	2.28	805.376
Calcium (mg kg^{-1})	2.88	18.441
Magnesium (mg kg^{-1})	2.17	13.936
Ash (%)	Na	23.9

nd denotes not determined, na denotes not applicable.

Changes in growth attributes and biochemical constituents of each tree species, and physical and chemical transformations that occurred in the growing media (garden soil or garden soil-biochar mixture) induced by each treatment, were measured every month for seven months.

Soil Analysis

In the text, 'samples' refer to either the garden soil or the soil-biochar mixture collected for analysis. On each sampling date, three samples were collected in Kopecky rings (diameter = 4.8 cm, height = 3.0 cm, volume = 55.0 cm) and weighed immediately to obtain fresh weights. Samples were oven dried in steel crucibles at 105 °C to constant weight to obtain dry weights. Based on the volume of the Kopecky ring and the change in the fresh and dry weights of the sample, bulk density (Bd) and particle density (Pd) were obtained (Ozcimen and Karaosmanoglu 2004). From the bulk density and particle density data, the total porosity (Tp) of the sample was calculated as follows.

$$Total\ Porosity\ (\%) = 1 - \frac{Bulk\ density}{Particle\ density} \times\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.1$$

From each pot described above, an extra 2 kg sample was collected, mixed thoroughly, and air-dried for three days on laboratory benches and used to evaluate the soil physical and chemical properties. The fractions of sand, clay, and silt particles in the 200 g sample were assessed according to Bouyoucous hydrometer method. About 50 g sample, placed in a crucible of known weight, was oven-dried at 105°C overnight to quantify available moisture content (AMC). The available moisture content in the sample was calculated as

$$Sample\ Moisture\ Content\ (\%) = \frac{Wet\ sample - ovendried\ sample\ (g)}{Wet\ sample\ (g)} \times 100 \ldots\ldots eq.2$$

About 200 g sample was saturated using a perforated bucket immersed in dish-containing water. The bucket and its contents were clamped on a retort stand overnight to allow drainage. The wet sample was transferred into a pre-weighed container (W1), and the container-containing wet sample gave the W2. The content was then oven-dried at 105 °C to constant weight to obtain dried weight (W3). Water-holding-capacity of the samples was calculated as

$$Water\ Holding\ Capacity\ (\%) = 100 \times \frac{(w2 - w1) - (w3 - w1)}{w3 - w1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.3$$

Where w1, w2, and w3 are the weights of the container, wet sample and container, and oven-dried sample and container.

For each sample collected, pH was determined in a 1:5 soil: water (v/v) suspension using JENWAY 3520 electrode pH meter (Mclean 1982); organic matter content was determined by loss on ignition method; organic carbon content was quantified by following Walkley and Black procedures while percentage nitrogen was quantified by following micro-Kjeldahl titration procedures of AOAC (1990). After digestion in H2SO4, available calcium (Ca) and magnesium (Mg) in each sample were determined in an atomic absorption spectrophotometer, and available potassium (K) and sodium (Na) were measured in a flame photometer (Ademiluyi and Omotoso 2008). Available phosphorous (P) was extracted by following the procedures of Brays (Bray's extraction method (No. 1), and P was determined colorimetrically (Bray and Kurtz 1945).

Plant Analysis

From each treatment, three seedlings were selected to monitor the changes in growth attributes of each tree species. The water applied in each pot was recorded, and the average of three measurements was calculated as the species' water demand on each sampling date. The pot and seedlings sampled above were weighed before and after watering. The average difference between the weight-before-watering and the weight-after-watering of three seedlings gave the species water use (PWU). The number of leaves on each plant was counted, and stem height and diameter were measured using the metric rule and Vernier big calipers. Owing to differences in the leaf shape of the evaluated trees species, the leaf area (LA) of *D. regia, V. merrillii,* and *D. lutescens* was calculated using the formula in Equation 4

$$LA = Leaf\ length\ \times Leaf\ width\ \times 0.5 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.4$$

LA of *B. monandra* was calculated as

$$LA = leaf\ length\ \times leaf\ width\ \times 0.78 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.5$$

Owing to its entire leaf shape, the LA of *T. catappa* was calculated as

$$LA = Leaf\ length\ \times Leaf\ width \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.6$$

The total leaf area (TLA) of each species was derived from the formula in Equation 7

$$Total\ leaf\ area = \frac{Number\ of\ leaves\ \times leaf\ area}{2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.7$$

After careful harvest, the roots of three seedlings from each treatment were rinsed separately five times in a bowl of water, and the lengthiest root of the seedling was measured to obtain root length. After separating each seedling into parts, fresh and dry weights (oven dried at 80+2 °C to constant weight) were obtained after weighing the root, stem, and leaf on a Mettler Toledo balance (MS1003S). From the dry weights obtained above, the root: shoot ratio (RSR) was calculated using Equation 8

$$Root{:}Shoot\ Ratio\ (RSR) = \frac{Dry\ Weight\ of\ Root}{Dry\ Weight\ of\ Stem + Dry\ Weight\ of\ Leaf} \ldots\ldots\ldots\ldots\ldots eq.8$$

Similarly, water use efficiency (WUE) was evaluated from total biomass and the total amount of water applied to each seedling, and the average of three measurements was assessed as follows.

$$Water\ Use\ Efficiency\ (g/L) = \frac{Total\ Biomass\ of\ seedling\ (g)}{Total\ Amount\ of\ Water\ applied\ (L)} \ldots\ldots\ldots\ldots\ldots\ldots eq.9$$

Relative water content (RWC) was determined in five fully expanded leaves of each species sampled from each treatment. Each leaf was corked (radius = 1.0 cm) at the base, middle,

and apex, and fresh weight was noted. The corked leaves were immersed in a cup of water for 24 hours to obtain turgid weight and then oven-dried at 65+2 °C until constant weight to derive dry weight. RWC was calculated as

$$Relative\ Water\ Content\ (\%) = 100 \times \frac{Fresh\ Weight - Dry\ Weight}{Turgid\ Weight - Dry\ Weight} \ldots\ldots\ldots\ldots\ldots eq.10$$

Chlorophyll and carotenoid were extracted from the leaves of each tree species collected from the treatments. About 0.5 g sample was macerated in 10 mL 80% acetone using a mortar and pestle. The mixture was centrifuged at 4000 rpm for 15 minutes, debris was discarded, and absorbance of the supernatant read at 664, 647, and 470 nm. The concentration of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), and carotenoid in the sample was calculated using the formulae of Lichtenthaller (1987). The specific leaf area (SLA) of a tree species in the treatments was calculated as the ratio of the leaf area to its dry matter, and the leaf area ratio (LAR) was calculated as leaf surface area (functioning photosynthesis) per unit dry weight of a seedling. The plant growth rate (PGR) of a tree species in the treatments was calculated as a relative increase in total biomass over time, and the relative growth rate (RGR) was calculated as the rate of accumulation of new biomass per unit of existing dry biomass.

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Proline content in the fresh leaves collected from the treatments was measured by following the ninhydrin-acetic acid method of Bates et al. (1973). About 20 mg leaf sample was extracted in 1 dm3 of 3% aqueous sulfosalicylic acid. The homogenate was centrifuged at 11500 rpm for 15 min using a TGL 16 electric centrifuge at 4 °C. Approximately 2 mL glacial acetic acid and 2 mL acidic ninhydrin reagent was added to 2 mL of the supernatant in the reaction tube, and the mixture was left to boil for 60 min in a water bath. After cooling on ice, about 4 mL of toluene was poured into the tube, and the reaction tube was incubated at room temperature for 30 min. After being shaken for 15 s, the tubes were left to stand for 10 min to separate the phases, and the upper phase was decanted in a clean test tube. The absorbance of the filtrate was measured at 520 nm using a spectrophotometer (UV-VIS 752N). Toluene was used as blank while free proline (L-proline - Sigma, Milan, Italy) was the standard reference. A standard curve was drawn by replacing the filtrate with different concentrations (between 0.04 - 1 mM) of standard proline in the procedures described above, and proline content in the sample was extrapolated from the curve.

The ascorbic acid content in the fresh leaves collected from the treatments was determined by following the 2, 6-dichlorophenol indophenol method described by AOAC (2000). About 200 mg leaf sample was macerated in 10 mL of 0.4% oxalic acid using a mortar and pestle. The mixture was centrifuged at 4000 rpm for 5 min, filtered, and the filtrate was collected in a clean test tube. About 2.5 mL filtrate was added to 5 mL of the activation mixture in an Erlenmeyer flask, and the solution was titrated against 2, 6-dichlorophenol indophenol dye. The volume of dye required to neutralize the acid was noted. Ascorbic acid was standardized by replacing the supernatant with different concentrations of standard ascorbic acid solution. Ascorbic acid was calculated and expressed in milligrams per 100 g fresh weight (mg 100g-1 FW).

Before the total phenolic acid (TPC) and flavonoid (TFC) contents analyses, the leaves were air-dried under shade and homogenized using a kitchen blender. About 1 g of the blended sample collected from each regime was extracted in 40 mL 90% aqueous methanol in a tightly closed 100 mL bottle. After shaking on Thomastant T-N22S (Thomas Kagaku Co. Ltd., Japan) for 1 hour, the content was filtered and concentrated in a rotary evaporator. The concentrated sample was stored in a clean bottle for later use. Total phenolic acid content was measured by following the Folin-Ciocalteu reagent method described by Sarker and Oba (2018). Approximately 1 mL Folin-Ciocalteu reagent (1:4 = 1 part reagent to 4 part distilled water) was added to 8 mL of concentrated extract in a test tube, mixed vigorously, and left to stand for 3 min. About 1 mL of 10% sodium carbonate was added, the solution was left in the dark for one hour, and absorbance was read at 760 nm on a spectrophotometer. Gallic acid was standardized by replacing the concentrated extract with different concentrations of standard gallic acid in the procedures described above and TPC in the leaf sample was extrapolated from the standard gallic acid plot. The TPC was expressed as gram gallic acid equivalent per gram dry weight (g GAE g−1 DW). The total flavonoid (TFC) in the concentrated sample was determined by following the aluminum chloride colorimetric method described by Sarker and Oba (2018). Approximately 1.5 mL of methanol, 0.1 mL of 1M potassium acetate, 0.1 mL of 10% aluminum chloride, and 2.8 mL of distilled water were allowed to react with 0.5 mL of concentrated extract in a test tube. The mixture was sonicated for 10 min, left to stand at room temperature for 30 min, and filtered. The absorbance of the filtrate was read at 510 nm using a spectrophotometer. Different concentrations of quercetin were substituted for extract in the above procedures, and a standard quercetin curve was drawn. The leaf flavonoid content was extrapolated from the curve, and the TFC was expressed as gram quercetin equivalent per gram dry weight (g QE g−1 DW).

Activities of SOD, CAT, and GPOx in the leaves sampled from each treatment were determined by following the procedures described by Giannopolitis and Ries (1977), Aebi and Lester (1984), and Rao et al. (1996) respectively. Harvest of fresh leaf samples for enzymatic antioxidants analysis was carried out at two-month intervals (precisely on 28th December 28, 2020, and on February 28, April 28, and June 28, 2021). About 0.5 g of leaf sample was collected on ice and homogenized in 10 mL ice-cold extraction buffer containing 9 mL of 0.2 M potassium phosphate (pH 7.0) and 1 mL of 0.1 M EDTA using a pre-chilled mortar and pestle. The homogenate was centrifuged at 15000 rpm for 10 min at 4 °C, and the supernatant was the enzyme extract. The enzyme extract was stored on ice until use. The SOD activity as induced by the treatments was measured in a 4 mL reaction mixture. The reaction mixture contained 50 µL enzyme extract, 500 µL EDTA (75 mM), 1 mL riboflavin (1.3 M), 950 µL (50 mM) phosphate buffers, 500 µL methionine (13 mM), and 1 mL nitro blue tetrazolium NBT (50 M). Reaction

commenced by switching on 15 W white lamps. A similar reaction mixture placed in the dark served as control, and a similar reaction mixture without enzyme extract served as blank. Photochemical repression of NBT by SOD in the enzyme extract was evaluated by taking the absorbance of the sample, blank, and control at 560 nm. One unit of SOD activity was defined as one enzyme unit per fresh weight (FW) of the sample that caused 50% inhibition of the photochemical reduction of NBT (assuming ε = 61.837 mM -1 cm -1 as the extinction coefficient of NBT at 560 nm).

Catalase activity (CAT, EC. 1.11.1.6) was measured in a 3 mL reaction mixture containing 0.1 mL enzyme extract, 2 mL potassium phosphate buffer (50 mM; pH 7.0), and 0.9 mL H2O2 (10 mM). The decomposition of H2O2 was monitored as a decrease in absorbance (every 30 s for 5 min) at 240 nm. Catalase activity was expressed as millimole H2O2 per min per gram fresh weight (mmol min-1 g−1 FW), assuming ε = 40 mM-1 cm−1 as the extinction coefficient of H2O2 at 240 nm. Similarly, GPOx activity was determined in a 3 mL reaction mixture containing 2.7 mL of potassium phosphate buffer (50 mM; pH 7.0), 0.1 mL of guaiacol (16 mM), and 0.1 mL enzyme extract. The reaction commenced by adding 0.1 mL of H2O2 (40 mM) to the reaction tube, and the absorbance was read at 470 nm on a digital spectrophotometer (UV-VIS 752N). Oxidation of guaiacol (blue color) to tetra guaiacol (purple color) was monitored by recording changes in absorbance at 15 s intervals for 2 min, and GPOx activity was estimated as a 0.01 unit increase in absorbance (assuming ε = 26.6 mM−1 cm−1 as the extinction coefficient of H2O2 at 470 nm). GPOx activity was expressed as millimole tetra guaiacol formed per min per milligram fresh weight (mM min-1 mg−1 FW).

The leaf total soluble carbohydrate content in the leaves from each treatment was determined according to the Anthrone principle. Approximately 50 mg fresh leaf sample was boiled in a test tube, hydrolyzed with 5 mL of 2.5N hydrochloric acid in a boiling water bath for 3 hours, and cooled to room temperature. The solution was neutralized by adding solid sodium carbonate until bubbles were formed, and distilled water was added until the volume reached 50 mL. The mixture was then centrifuged at 4000 rpm for 10 min at room temperature, and the supernatant was collected in a clean test tube. About 1 mL supernatant was added to 4 mL Anthrone reagent in a thick-walled test tube, heated in a boiling water bath for 8 min, and then cooled to room temperature. The absorbance of the solution was read at 630 nm using a spectrophotometer (UV-VIS 752N). Glucose was used as a reference standard, and water was used as a blank. Glucose stock solution was prepared by dissolving 0.1 g glucose in 100 mL distilled water, and the working standard was prepared from 10 mL glucose stock solution. About 0, 0.2, 0.4, 0.6, 0.8, and 1 mL of the working standard were poured into 6 separate test tubes and diluted with distilled water until the volume reached 1 mL. A standard curve of L-glucose was drawn by replacing the supernatant with working standards in the procedures described above, and glucose concentration in the sample was extrapolated from the standard curve. The total soluble carbohydrates in the sample were calculated as

$$Total\ soluble\ Carbohydrate\ (mg/100g) = \frac{Mass\ of\ glucose}{Volume\ of\ test\ sample} \times 100 \ldots\ldots\ldots eq.11.$$

Protein content in the leaves sampled from each treatment was determined by following the micro-Kjeldahl nitrogen method described in AOAC (2000). The percentage of crude protein was estimated using the formulae below

$$Nitrogen\ (\%) = \frac{(A - B) \times N \times 14.01 \times 100}{1\ gram\ of\ sample \times 10} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.12$$

$$Crude\ Protein\ (\%) = \%\ Nitrogen\ \times 6.25 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.13$$

A = sample reading, B = blank reading; N = normality of the acid used for the titration, 100 = % conversion, and 6.25 is the correction factor (F).

Statistical Analysis

All experiments were carried out in triplicate, and sampling was carried out every month for seven consecutive months except where otherwise stated. The data obtained were analyzed by using SPSS version 16. Soil property, tree growth attribute, and biochemical constituent were analyzed using a univariate general linear model (GLM), and treatment means were separated using Tukey's Honest Significant Difference (HSD) at $P<0.05$. For soil physical and chemical properties, the data mean in the control regime (T1 treatment) was compared to data means in the other treatment regimes (i.e., T2, T3, T4, T5, and T6). The data of each soil property, tree growth attribute, and biochemical constituent were pooled separately according to the treatment (T1, T2, T3, T4, T5, and T6), and the data mean of the T1 treatment was compared to those of the other treatments.

RESULTS

Single and interactive effects of Irrigation Rate and Sawdust Biochar on soil physical and chemical properties

Table 2 shows that irrigation of garden soil at 60% FC (T2 treatment) and 35% FC (T3 treatment) and irrigation of soil-biochar mixture at 35% FC (T6 treatment) decreased the fraction of sand and clay particles but increased the fraction of silt particles. In contrast, irrigation of soil-biochar mixture at 80% FC (T4 treatment) and 60% FC (T5 treatment) caused a transient increase in the fraction of sand and clay particles but slightly decreased the fraction of silt particles compared to irrigation of garden soil at 80% FC (control or T1 treatment).

Table 2: Effects of irrigation rate and sawdust biochar on physical and chemical properties, and available macronutrients of soil and soil-biochar mixture used for growing the five tree species investigated

Properties/ Treatments	T1 (Control)	T2	T3	T4	T5	T6
Sand (%)	66.608^{ab}	66.581^{ab}	66.365^{b}	67.025^{a}	66.942^{ab}	66.492^{ab}
Clay (%)	15.442^{a}	15.437^{a}	15.421^{a}	15.516^{a}	15.475^{a}	15.430^{a}
Silt (%)	17.950^{a}	17.982^{a}	18.214^{a}	17.459^{a}	17.583^{a}	18.078^{a}
Bulk Density (g cm^{-3})	1.398^{c}	1.434^{abc}	1.458^{a}	1.189^{e}	1.263^{d}	1.405^{bc}
Particle Density (g cm^{-3})	2.059^{ab}	2.074^{a}	2.109^{a}	1.844^{c}	1.869^{bc}	2.091^{a}
Total Porosity (%)	47.233^{c}	45.881^{cd}	45.000^{d}	55.126^{a}	52.358^{b}	46.981^{cd}
Available Moisture Content (%)	34.945^{c}	34.599^{d}	34.140^{e}	37.620^{a}	37.367^{a}	35.611^{b}
Water Holding Capacity (%)	42.022^{c}	41.698^{c}	40.865^{c}	45.131^{ab}	45.734^{a}	44.035^{b}
pH	6.026^{b}	5.998^{b}	5.977^{b}	6.667^{a}	6.618^{a}	6.583^{a}
Electrical Conductivity (µS cm^{-1})	67.667^{c}	64.917^{c}	63.833^{c}	97.606^{a}	89.500^{b}	85.417^{b}
Organic Matter (%)	4.471^{d}	4.327^{d}	3.962^{d}	22.162^{a}	17.026^{b}	12.961^{c}
Organic Carbon (%)	2.039^{d}	1.665^{d}	1.622^{d}	12.864^{a}	10.135^{b}	7.329^{c}
Total Nitrogen (%)	0.172^{d}	0.135^{d}	0.122^{d}	0.494^{a}	0.373^{b}	0.266^{c}
C:N ratio	11.651^{b}	12.519^{b}	13.187^{b}	26.059^{a}	27.111^{a}	27.848^{a}
Phosphorus (mg kg^{-1})	7.417^{cd}	6.893^{cd}	6.696^{d}	9.280^{a}	8.578^{ab}	7.752^{bc}
Potassium (mg kg^{-1})	1.258^{d}	1.213^{d}	1.207^{d}	17.747^{a}	16.201^{b}	14.748^{c}
Calcium (mg kg^{-1})	1.898^{d}	1.729^{d}	1.537^{d}	48.542^{a}	43.400^{b}	34.974^{c}
Magnesium (mg kg^{-1})	1.277^{c}	1.202^{c}	1.083^{c}	7.770^{a}	6.773^{ab}	5.846^{b}

Values along the row bearing same letters are not significantly different at $\alpha = 0.05$ level of significance.

T1, T2 and T3 were soil irrigated at 80%, 60% and 35% FC respectively, T4, T5 and T6 were soil-biochar mixture irrigated at 80%, 60% and 35% FC respectively

Without biochar application, Bd of 35% FC-irrigated soil increased significantly by 4.3%. In contrast, Bd decreased in the presence of biochar by 9.7 and 4.9% at 80% and 60% FC irrigation rates. Similarly, the particle density of 80% FC-irrigated soil decreased significantly by 10.4% in the presence of biochar. Total porosity (Tp) of 80% and 60% FC-irrigated soils increased by 16.7 and 10.9% with biochar application, and Tp decreased by 4.7% in the 35% FC-irrigated soils. In addition, available moisture content (AMC) increased significantly by 7.7, 6.9 and 1.9% in the 80%, 60%, and 35% FC-irrigated soils, and AMC of the 60% and 35% FC-irrigated soils decreased significantly by 1.0 and 2.3% respectively. In addition, in the presence of biochar, water-holding capacity (WHC) of 80%, 60%, and 35% FC-irrigated soils increased by 7.4, 8.8, and 4.8%. Compared to 80% FC-irrigated soils, pH and electrical conductivity (EC) reduced transiently by 0.8 and 5.7% in the 35% FC-irrigated soils and by 0.5 and 4.1% in the 60% FC-irrigated soils. In contrast, pH increased significantly by 10.6, 9.8, and 9.2%, and EC increased by 44.2, 32.3, and 26.2% in the 80%, 60%, and 35% FC-irrigated soils with biochar application. Similarly, 60% and 35% FC irrigation rates caused transient decreases of 3.2 and 11.4% in organic matter content, 18.3 and 20.5% in organic carbon content, and 21.5 and 29.1% in percentage nitrogen of soils (Table 2). However, in the presence of biochar, 80%, 60%, and 35% FC watering rates caused significant increases of 187.2, 116.9 and 54.7% in percentage nitrogen, 395.7, 280.9, and 189.9% in organic matter contents, and organic carbon content of soil increased significantly by 530.9, 397.1 and 259.4% respectively. Soil carbon: nitrogen ratio (C/N) decreased transiently by 7.5 and 13.2% in the 60% and 35% FC-irrigated soils but increased significantly by 123.7, 132.7 and 139.0% in soil-biochar mixture irrigated at 80%, 60% and 35% FC respectively (Table 2).

Extractable macronutrients in the samples varied widely depending on the treatment. Relative to 80% FC irrigation treatment, the application of 60% FC irrigation decreased the extractable P, K, Ca, and Mg slightly by 7.1, 3.6, 8.9 and 5.9%, and the application of 35% FC irrigation caused transient decreases of 9.7, 4.1, 19.2 and 15.2% in the extractable P, K, Ca, and Mg respectively. However, in the presence of biochar, extractable P, K, Ca, and Mg increased sporadically by 25.1, 1310.7, 2457.5, and 508.5% in the 80% FC irrigation treatment and by 15.7, 1187.8, 2186.6 and 430.4% in the 60% FC irrigation treatment. Similarly, in the presence of biochar, dissolved K, Ca, and Mg were 1072.3, 1742.7, and 357.8% higher in the 35% irrigated soils.

Single and interactive effects of irrigation rates and sawdust biochar on growth attributes and biochemical constituents of the evaluated trees species

From Figure 1, the water demand of the five tree species varied widely from one treatment to another. On average, irrigation demand of trees seedlings in the 80% FC-irrigated soils (control) was 7.904 liter per month, a value that was 15.3 and 31.7% higher than the irrigation demand of 60% and 35% FC-irrigated seedlings grown without biochar; and 5.5, 17.6 and 33.6% higher than 80%, 60% and 35% FC-irrigated seedlings grown in biochar-treated soils. Similarly, water use of 60% and 35% FC-irrigated trees seedlings grown without biochar and those of 35% FC-irrigated trees seedlings grown in biochar-treated soils decreased significantly by 12.8, 24.5 and 14.3%, and biochar increased the water use of 80% and 60% FC-irrigated seedlings slightly by 4.2 and 7.3% compared to seedlings in the control regime.

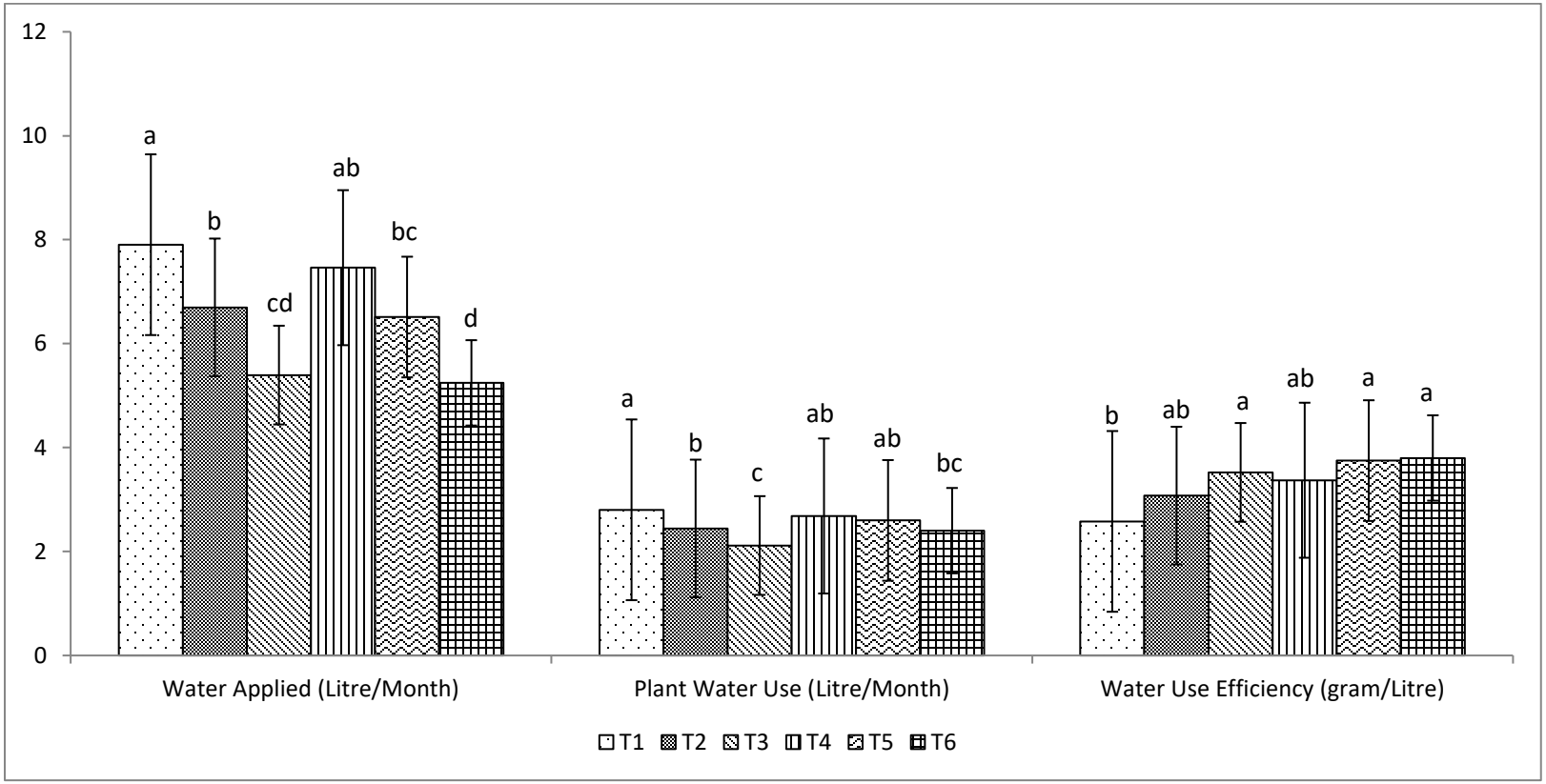

Figure 1: Effects of irrigation rate and biochar application on water applied, water use and water use efficiency of species of trees commonly used in urban landscaping in Southwest Nigeria

T1, T2 and T3 = tree species grown on soil and irrigated at 80, 60 and 35% FC respectively; T4, T5 and T6 = tree species grown on soil-biochar mix and irrigated at 80, 60 and 35% FC respectively. Three seedlings per species and three replicates per treatment. Vertical bars represent error bars with standard error.

Table 3: Effects of water application rates and sawdust biochar on the morphological attributes and biochemical constituents of seedlings of five tree species commonly used in urban landscaping in Ondo city, Nigeria

Treatments	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)	Plant Dry Weight (g)	Root: Shoot Ratio	Dickson Quality Index (g)
T1 (Control)	40.836^{bc}	10.207^{abc}	3.013^{b}	0.870^{bc}	1.134^{bc}	32.134^{a}	59.015^{ab}	22.401^{ab}	0.433^{a}	1.331^{a}
T2	38.241^{c}	9.106^{c}	2.940^{bc}	0.770^{bc}	0.906^{c}	31.554^{a}	53.553^{b}	21.071^{b}	0.438^{a}	1.304^{a}
T3	33.056^{c}	7.797^{c}	2.547^{c}	0.617^{c}	0.571^{c}	29.776^{a}	51.004^{b}	19.948^{b}	0.447^{a}	1.246^{a}
T4	53.231^{a}	13.135^{a}	3.687^{a}	1.152^{a}	2.022^{a}	34.226^{a}	71.677^{a}	27.365^{a}	0.422^{a}	1.521^{a}
T5	48.233^{ab}	12.401^{ab}	3.487^{a}	1.018^{ab}	1.711^{ab}	33.112^{a}	65.587^{ab}	25.737^{ab}	0.417^{a}	1.492^{a}
T6	37.639^{c}	9.939^{bc}	2.834^{bc}	0.728^{c}	0.907^{c}	29.982^{a}	56.851^{ab}	21.428^{ab}	0.434^{a}	1.269^{a}
Significance	**	**	**	**	**	ns	**	*	ns	*

Treatments	Superoxide dismutase (Unit min^{-1} $mg^{-1}FW$)	Guaiacol peroxidase $\times 10^{-4}$ ($\mu Mmin^{-1}$ $mg^{-1}FW$)	Catalase $\times 10^{-2}$ (μM min^{-1} $mg^{-1}FW$)	Proline (μM $g^{-1}FW$)	Total flavonoid (g $g^{-1}QE$)	Phenolic acid (g $g^{-1}GAE$)	Ascorbic acid (mM $g^{-1}FW$)	Total carbohydrate (mg $100mg^{-1}$)	Malondi-aldehyde $\times 10^{-3}$ (nM mL^{-1})	Crude protein (%)
T1 (Control)	9.005^{bcd}	7.762^{bc}	4.001^{d}	12.644^{c}	0.093^{d}	0.438^{c}	2.514^{a}	14.062^{b}	4.985^{a}	17.015^{b}
T2	9.481^{abc}	8.320^{b}	4.896^{c}	13.800^{c}	0.100^{c}	0.543^{b}	2.567^{a}	13.421^{b}	5.038^{a}	15.570^{c}
T3	10.214^{a}	9.877^{a}	6.333^{a}	18.667^{a}	0.113^{a}	0.603^{a}	2.914^{a}	11.185^{c}	5.061^{a}	13.454^{d}
T4	8.010^{d}	6.236^{d}	1.847^{f}	8.327^{d}	0.066^{f}	0.436^{c}	1.711^{b}	16.446^{a}	4.915^{a}	18.425^{a}
T5	8.607^{cd}	7.111^{c}	2.931^{e}	9.353^{d}	0.079^{e}	0.477^{c}	1.943^{b}	15.302^{a}	4.959^{a}	17.978^{ab}
T6	9.878^{ab}	9.128^{a}	5.630^{b}	16.061^{b}	0.107^{b}	0.579^{b}	2.783^{a}	11.765^{c}	5.003^{a}	14.730^{cd}
Significance	**	**	**	**	**	**	**	**	**	**

Values along the column bearing same letters are not significantly different at $\alpha < 0.05$.

T1, T2 and T3 = tree species grown on soil and irrigated at 80, 60 and 35% FC respectively; T4, T5 and T6 = tree species grown on soil-biochar mixture and irrigated at 80, 60 and 35% FC respectively. ** and * denote significance at $P<0.01$ and $P<0.05$ respectively. ns denotes no significant difference at $\alpha < 0.05$. For superoxide dismutase, guaiacol peroxidase and catalase activities, N = 60 (i.e. three seedlings per species per month for four months (December, 2020 and February, April and June 2021) and for other attributes, N = 105 (i.e. three seedlings per species per month for 7 months).

Interestingly, ANOVA revealed that water use efficiency (WUE) of 35% FC-irrigated tree seedlings was significantly higher by 36.6 and 47.3% with or without biochar than those seedlings grown in the control regime. In addition, WUE was 45.3% higher in the biochar-treated-60% FC irrigated seedlings but slightly higher by 19.2 and 30.7% in the 35% FC-irrigated seedlings and biochar-treated-80% FC irrigated seedlings at $P<0.05$. There were no significant changes in most of the measured morphological attributes of the 60% and 35% FC-irrigated tree seedlings compared with the 80% FC-irrigated seedlings. However, stem girth (SG) of the 35% FC-irrigated tree seedlings decreased significantly by 15.5%. In the presence of biochar, SG of 80% and 60% FC-irrigated seedlings increased by 22.4 and 15.7% more than those in the 80% FC-irrigated seedlings. Similarly, the application of sawdust biochar increased the shoot height (SH), LA, and TLA of trees' seedlings by 30.4, 32.4, and 78.3% relative to trees grown in the control regime (Table 3).

Biochemical constituents in the evaluated tree species varied widely depending on the treatment (Table 3). Compared to trees grown in the control regime, activities of SOD, GPOx, and CAT increased significantly by 13.4, 27.2 and 58.3% in the 35% FC-irrigated seedlings; CAT activity increased significantly by 22.4 and 40.7% in the 60% FC-irrigated seedlings and biochar-treated-35% FC irrigated seedlings respectively while GPOx activity was sped-up by 17.6% in the 35% FC-irrigated seedlings. In the presence of biochar, activities of GPOx and CAT decreased significantly by 19.7 and 53.8% in the 80% FC-irrigated seedlings, and CAT activity decreased by 26.7% in the 60% FC-irrigated seedlings. Leaf proline in the 35% FC-irrigated seedlings was higher by 27.0% and 47.6% with and without biochar, and biochar in the 80% and 60% FC-irrigated soil decreased the leaf protein by 34.1 and 26.0% compared to trees grown in the control regime (Table 3). Similarly, leaf TFC in the 35% FC-irrigated seedlings was higher by 21.5 and 15.1%, and leaf TPC in these seedlings was higher by 37.7 and 32.2% with and without biochar. In addition, leaf TFC and TPC increased by 7.5 and 24.0% in the 60% FC-irrigated seedlings. In the presence of biochar, leaf TFC, and ascorbic acid content were 29.0 and 31.9% lower in the 80% FC-irrigated seedlings and 15.1 and 22.7% lower in the 60% FC-irrigated seedlings. Total soluble carbohydrates (TSC) in the 35% FC-irrigated trees leaves were 20.5% and 16.3% lower, and leaf crude protein content (CP) was 20.9 and 13.4% lower with or without biochar. In addition, leaf CP was 8.5% lower in the 60% FC-irrigated seedlings. However, in the presence of biochar, leaf TSC, and CP were higher by 17.0 and 8.3% in the 80% FC-irrigated seedlings, and leaf TSC of the 60% FC-irrigated seedlings was 8.8% higher compared to the seedlings in the control regime. Interestingly, accumulated MDA in the leaves of the tested trees was not significantly affected, such that the MDA content of the control seedlings was not different from those in the other regime at $P<0.05$.

Physiological attributes - PGR, RGR, LAR, and net assimilation rate (NAR) of seedlings in the control regime were statistically similar to those in other treatment regimes. However, the SLA of 35% FC-irrigated seedlings decreased significantly by 31.5 and 21.9% with or without biochar (Table 4). In the presence of biochar, leaf RWC of 80% FC-irrigated seedlings was significantly higher by 4.4% compared with seedlings in the control regime. Accumulated Chl a, Chl b, and Chl a+b were 12.3, 18.0, and 14.9% lower in the 35% FC-irrigated seedlings than in the control seedlings. In the presence of biochar, accumulated Chl b increased significantly by 17.3 and 12.9%, and Chl a+b content increased by 8.8 and 6.4% in the 80% and 60% FC-irrigated seedlings compared to the control seedlings.

Table 4: Effects of sawdust biochar and water application rates on physiological attributes of seedlings of five tree species commonly used in urban landscaping in Ondo City, Nigeria.

Treatments	Relative Water Content (%)	Plant Growth Rate (g day^{-1})	Relative Growth Rate × 10-2 (g day^{-1})	Leaf Area Ratio (g cm^{-2})	Specific Leaf Area (g cm^{-2})	Net Assimilation Rate × 10-3 (g day^{-1})
T1 (Control)	70.460^{bc}	0.201^{ab}	1.393^{a}	5.110^{abc}	667.338^{a}	1.378^{a}
T2	69.854^{bc}	0.189^{ab}	1.362^{a}	4.857^{bc}	521.098^{c}	1.393^{a}
T3	68.132^{c}	0.168^{b}	1.305^{a}	4.603^{c}	456.847^{c}	1.447^{a}
T4	73.568^{a}	0.239^{a}	1.627^{a}	5.914^{a}	728.312^{a}	1.429^{a}
T5	72.442^{ab}	0.224^{ab}	1.523^{a}	5.641^{ab}	702.318^{a}	1.439^{a}
T6	69.139^{c}	0.181^{b}	1.342^{a}	4.889^{bc}	603.565^{ab}	1.383^{a}
Significance	**	*	ns	**	**	ns

Treatments	Chlorophyll a (µM g^{-1}FW)	Chlorophyll b (µM g^{-1}FW)	Total chlorophyll (µM g^{-1}FW)	Chlorophyll a/b ratio	Carotenoid (µM g^{-1}FW)	Carotenoid: Chlorophyll ratio
T1 (Control)	16.442^{ab}	13.458^{b}	29.901^{ab}	1.337^{a}	4.097^{ab}	0.178^{bc}
T2	15.558^{bc}	12.339^{bc}	27.898^{bc}	1.381^{a}	4.428^{ab}	0.201^{ab}
T3	14.423^{c}	11.029^{c}	25.452^{c}	1.505^{a}	4.582^{a}	0.225^{a}
T4	17.956^{a}	15.785^{a}	32.522^{a}	1.493^{a}	3.336^{d}	0.129^{d}
T5	17.522^{a}	15.191^{a}	31.826^{a}	1.477^{a}	3.459^{cd}	0.134^{cd}
T6	15.607^{bc}	12.806^{b}	28.413^{b}	1.292^{a}	3.989^{bc}	0.176^{bc}
Significance	**	**	**	ns	**	**

Values along the column bearing same letters are not significantly different at P<0.05;

Values are the mean ± standard error. T1, T2 and T3 = tree species grown on soil and irrigated at 80, 60 and 35% FC respectively; T4, T5 and T6 = Tree species grown on soil-biochar mixture and irrigated at 80, 60 and 35% FC respectively. ** and * denotes overall mean of treatment significant at P<0.01 and P<0.05 respectively. For net assimilation rate, plant and relative growth rate, N = 90 (i.e. three seedlings per species per month for six months (7-1 months), and for other attributes, N = 105 (i.e. three seedlings per species per month for 7 months).

Similarly, biochar in the 80% and 60% FC-irrigated soils decreased the carotenoid content by 18.6% and 15.6% compared to the control seedlings. The ratio of carotenoid/ chlorophyll in the five landscape tree species was 26.4% higher in the 35% FC-irrigated seedlings but 27.5% lower in the biochar-treated-80% FC irrigated seedlings (Table 4).

DISCUSSION

Single and interaction effects of water application rate and sawdust biochar on physical and chemical properties of soil and soil-biochar mixture

From the results presented above, 80%, 60%, and 35% irrigation rates had no significant impact on soil texture. Likewise, the textural class did not change significantly in the biochar-treated 80%, 60%, and 35% FC-irrigated soils. Therefore, soil textural change cannot be responsible for the morphological, physiological, and biochemical changes observed in the tree seedlings under investigation. However, sawdust biochar decreased the Bd and Pd but enhanced the Tp and AMC of the 80%, 60%, and 35% FC-irrigated soil. These transformations eased rhizospheric root penetration and regulated water and nutrient acquisition in the 80% and 60% FC-irrigated seedlings. Therefore, the growth of tree seedlings in these regimes was luxuriant and of high visual quality. Prendergast-Miller et al. (2014) attributed the improvement in the plant's nutrient uptake and acquisition to biochars' ability to attract and guide fine and coarse roots towards available nutrients and their ability to partition the bulk from rhizosphere soil.

In contrast, restricted water and air transport and a preferential flow path formed from repeated wetting and drying could be responsible for the bulkiness, high particle density, low porosity, and available moisture content of 35% FC irrigated soil. Peng et al. (2007) discovered that pore shrinkage induced by wetting and drying cycles could hinder the free flow or transport of water and air in the soil. Korenkova and Urik (2012) reported that Bd increased while Tp, wettability, and infiltration rate decreased significantly in water-stressed soil. Thus, as soil desiccation increased, misconfiguration of structural pores deformed the soil matrix, and this ultimately led to poor growth and visual performance of the 35% FC-irrigated tree seedlings. Similarly, decreasing trends of soil organic matter and carbon contents as irrigation rates decreased indicate that solubility, mineralization, and decomposition of organic matter depend on the soil water level. Although Magid et al. (1999) observed no significant change in the decomposition rate of complex substrates (organic matter and carbon) in drought conditions, Lomander et al. (1998) observed a linear relationship between the decomposition rate of organic matter and soil moisture content while Andersson et al. (2000) attributed the low level of dissolved carbon in the soil to the significant change in charge density of humic compounds caused by the low solubility of organic matter in water stress condition. Recently, Sardans et al. (2008) and Badiane et al. (2012) confirmed that solubility and mineralization of soil organic matter decreased with decreasing soil moisture. However, soil acidity tended to increase with decreasing irrigation rates, as reflected in the highest and lowest pH observed for the 80% and 35% FC irrigated soils, respectively. This result could be associated with reduced solubility and mineralization of organic matter, decreased nitrification and mineralization of nitrogen, and repressed soil microbial activities in the 35% FC-irrigated soils.

However, a transient decrease in the percentage nitrogen of 35% FC irrigated soil suggests an effective transformation of atmospheric nitrogen and native ammonia to plant-

available nitrogen. In other words, the physiological activity of soil nitrifying and nitrogen-mineralizing microbes, the rate of conversion of ammonia (oxidation) to NH4-N or NO3-N in the soil, and their passive transport into the tree tissues grown in the 35% FC irrigation rate were at par with those seedlings in the 80% FC-irrigated seedlings. Soil moisture deficit performs a vital role in regulating the mineralization of soil nitrogen (Neina 2019).

Similarly, there was no hindrance to the solubility and mobility of cations in the 60% and 35% FC- irrigated soils, as reflected in slightly lower concentrations of extractable macronutrients in these regimes compared to the 80% FC-irrigated soils. Such free flow and solubilization could ensure nutrient availability, uptake, and acquisitions by the 60% and 35% FC-irrigated seedlings. It can also account for similar growth rates and biochemical constituents of the seedlings in all regimes. Since texture, percentage nitrogen, and extractable cations in the 35% FC irrigated soils were at parity with those in the 80% FC irrigated soils, then irrigation of trees seedlings at 35% FC rate is seemingly a promising technique for conserving municipal water resources and nutrients input in the nurseries.

Impacts of biochar on physicochemical properties of soil irrigated at different rates

Biochar properties such as high porosity, cations exchange, nitrogen retention capacity, and oxygenated and carboxyl groups on biochar surfaces may improve the physical and chemical properties of the soil-biochar mixture, especially at 80% and 60% FC irrigation regimes. These properties may improve the soil properties in the 35% FC irrigation rate compared to 35% FC-irrigated garden soils without biochar. From the results above, biochar reduced the Bd but increased the Tp, AMC, WHC, pH, electrical conductivity, organic matter and carbon contents, and percentage nitrogen of the soil-biochar mixture. An increase in the porosity of pyrolyzed material due to the transformation of the preserved cell wall of feedstock during pyrolysis may lower the bulk density of biochar than those of its feedstock and soil (Downie et al. 2009; Major et al. 2009) and upon incorporation in the soil lower the Bd by increasing the soil pore volume (Lehmann 2007). This observation possibly explains the lower Bd of the soil-biochar mixture than that of garden soil, especially in the 80% and 60% FC irrigation regimes. In addition, the higher AMC in the 80% FC and 60% FC-irrigated soil-biochar treatments than in the garden soil alone could be due to the high porosity of biochar that allowed it to act like a sponge, capable of absorbing and retaining soil moisture (Thies and Rillig 2009).

Alternatively, an increase in the Tp and AMC of the soil-biochar mixture could be due to carboxyl groups on the biochar's surface. Carbonyl groups on biochars' surfaces can functionally aid the binding of soil micro-aggregates (particles) to form macro-aggregates (Downie et al. 2009; Shackley and Sohi 2010), and such rearrangement of soil particles often provides a large surface area for intermolecular attractions between biochar and water molecules. As soil dried up, the additional water stored in the micro-pores of sawdust biochar gradually flowed out and, as a consequence, alleviated water-induced stress via increasing the solubility and mobility rates of nutrients and uptake and transportation of nutrients and water in the 60% and 35% FC-irrigated trees seedling. Previous studies recorded higher AMC and WHC in biochar-treated sandy soil than in bare soil (Ruehr 2007; Uzoma et al. 2011; Basso et al. 2013) and Bordoloi et al. (2018) reported the same results under drought conditions. Similarly, after incubation for 168 days, Hseu et al. (2014) found that Tp and WHC were significantly higher in the biochar-treated mudstone soils than in the untreated ones.

Oxygenated functional groups found on biochar's surfaces can increase the abundance of soil exchangeable base cations and base saturation by binding soil aluminum ions (Al3+) and soluble iron (Fe) (Yuan et al. 2011). Therefore, oxidation of soil Al3+ and soluble Fe by sawdust biochar could have resulted in higher pH of the soil-biochar mixture than in the garden soils. Alternatively, 23.9% ash content shows that sawdust biochar was very rich in ash, and accretion of ash possibly increased the pH of the soil-biochar mixture compared to that of the garden soils. In other words, like biochar derived from other organic feedstocks, sawdust biochars ash contained mixtures of carbonates of alkali and alkaline earth metals, relatively high phosphates, organic and inorganic nitrogen, and abundant 'basic' charged (organic anions) groups (Khanna et al. 1994; Arocena and Opio 2003; Agusalim et al. 2010; Yu et al. 2012) which aided neutralization of garden soil acidity. Furthermore, the slightly acidic, near to neutral pH of the soil-biochar mixture favored microbial degradation and solubility of organic matter and carbon, which as a consequence, jerked up organic matter and carbon contents in the biochar-grown 80%, 60%, and 35% FC-irrigated seedlings. Besides, Hamer et al. (2004) revealed that adequate temperature, aeration, moisture, and nitrogen content are essential abiotic factors for the rapid decomposition of litter in forest soil. Thus, higher organic matter and carbon contents could be due to high AMC that sped up organic matter degradation and solubility in the soil-biochar mixture. Gradual release of additional water from biochar micro-pores as the soil dries up maintained the rate of organic matter degradation and dissolution of native soil carbon in the soil-biochar mixture.

Similarly, a higher nitrogen percentage in the soil-biochar mixture than in the garden soil could be due to the capacity to retain soil nitrogen and absorb toxicants, high porosity, specific surface area (mainly for anionic adsorption), and liming effect of biochar. These biochars' properties may aid the activities of nitrifying bacteria, reduce leaching, and increase stored nitrate ions in the soil (Mukherjee et al. 2013). Novak et al. (2010) observed that nitrogen mineralization increased significantly following the biochar amendment of forest soil. Although the carbon: nitrogen ratio in the soil-biochar mixture was high - 26:1, 27:1, and 28:1 in the 80%, 60%, and 35% FC-irrigated soils, respectively - and sufficient to induce soil nitrogen immobilization, there was not a single symptom of nitrogen immobilization shown on trees seedlings grown in these regimes. This observation could be due to the high recalcitrance of biochar's carbon explained by Cox et al. (2012). The authors suggested that nitrogen immobilization (temporary loss or withdrawal of available nitrogen) may not occur in forest soil at 25-30 parts carbon to one part nitrogen because of the high half-life of biochar's carbon. They further stressed that this range of C/N ratio (25-30) was ideal for litter decomposition in forest soil.

In the 80% and 60% FC irrigation regime, sawdust biochar induced a slight increase in the extractable K, Ca, P, and Mg were significantly higher in the soil-biochar mixture than in the garden soil, especially at 80% and 60% FC irrigation rates. The following factors may be responsible for these observations: First, biochar made from wood waste (such as sawdust biochar) often contains high amounts of soluble K, P, and Ca, and when incorporated in the soil are released to promote plant growth (Page-Dumroese et al. 2015). Second, oxidation of carboxylate and other ionizable functional groups on biochar surfaces often promotes the absolute dissolution of cations like K, Ca, Mg, and even P in the native soil (Cheng et al. 2006). The near-to-neutral pH of the soil-biochar mixture seemed to favor the rates of mineralization

and solubility of K, Ca, and Mg labile on biochar (Prasad et al. 2018). Sawdust biochar also appeared to support the microbial transformation of phosphorous, nitrogen, and sulfur by turning their micro-pores into microhabitats for phosphate-solubilizing bacteria genera and microbes involved in N and sulfur (S) transformation. Pietikainen et al. (2000) reported a similar result. Lastly, liming potential of sawdust biochar (due to high ash content) increased and ensured rapid dissolution of Ca and Mg native in the soil.

Effects of water application rates on growth and biochemical constituents of five tree species commonly used in urban landscaping in Ondo, Nigeria.

Water is an essential component of plants. It is a critical, inevitable substance at every stage of plants' growth, hence the life wire of plants' growth and development. Water plays a vital role in all nursery operations, from germination to seedlings/saplings stage in the nursery pen and the establishment of transplants on the street. Water available for irrigation purposes determines to a large extent the quality of tree seedlings for urban landscaping. In the current study, water demand and water use were significantly higher for the 80% FC-irrigated seedlings than for the 60% and 35% FC-irrigated seedlings. However, water use efficiency was significantly higher for the 35% FC-irrigated seedlings than the 80% FC-irrigated seedlings. Although irrigation of the evaluated trees at 80% FC had the most satisfactory growth attributes, there were no significant differences in the morphological and physiological traits (SH, SG, LN, LA, TLA, LAR, fresh weight and biomass, RL, RSR, leaf RWC, PGR, RGR, LAR, NAR, and carotenoid content) of the 80% FC-irrigated seedlings and those of the 60% and 35% FC-irrigated ones. Simply 35% FC-irrigated tree seedlings showed relatively similar morphological and physiological characteristics and advantageous water use efficiency compared to the 80% and 60% FC-irrigated ones.

In addition, chlorophyll loss and carotenoid content were statistically similar in the 80% and 60% FC-irrigated seedlings, but chlorophyll loss was significantly higher in the 35% FC-irrigated seedlings. This result suggests that a 60% FC irrigation rate caused mild oxidative stress in the tree seedlings grown on it, and the carotenoid in the seedlings effectively preserved the functional integrity of photosystem II (PSII). According to McKinnon and Mitchell (2003), transient chlorophyll loss is a regulatory mechanism that reduces light harvesting and enhances photo-protection in water-stressed plants. Similarly, leaf RWC was statistically similar for the 80%, 60%, and 35% FC-irrigated seedlings, and this could be due to the availability and uptake of relatively equal concentrations of potassium (K+) by the tree seedlings in the three regimes. The similarity in the available K in the 80%, 60%, and 35% FC-irrigated soils might influence the potassium uptake of the seedlings in the three regimes and thus explain parity in their leaf turgidity since the primary function of K is to regulate plants water status. Potassium (K) functions in osmoregulation by promoting water absorption by the roots, keeping osmotic tension and turgor in the cells and plant tissues, and regulating the activity of stomata cells to prevent unnecessary water loss by transpiration.

However, the thin stem of the 35% FC-irrigated seedlings indicates that the tested trees are sensitive to water stress. This result is similar to previous reports that seedlings of *Brachystegia eurycoma, Picralima nitida, Vangueria infaustia,* and *Persea americana* showed poor growth attributes in water-stressed conditions (Ikojo et al. 2005; Gbadamosi 2014; Mng'omba et al. 2011). Lei et al. (2006) suggested that oxidative bursts may injure cellular

organelles, damage quaternary protein structure, fragment nucleic acid, and impair other physiological processes. Therefore, significant chlorophyll loss in the 35% FC irrigated seedlings was presumed to be associated with water-stressed-induced active oxygen species formation, and this possibly caused an oxidative burst and deformed the structure of the chloroplast. However, the lack of significant difference between the malondialdehyde (MDA) content of 80% FC-irrigated seedlings and those of 35% FC-irrigated seedlings showed that the latter treatment did not destroy the cellular organelles (chloroplasts) of the trees' seedlings. However, previous investigators observed a significant increase in the MDA content of *Jathropha curcas* (Silva et al. 2015), *Populus kangdingensis*, and *Populus cathayana* (Yin et al. 2015) seedlings in water-stressed conditions and symptomized it with the level of damage to cellular organelles. However, significant chlorophyll loss reflected in the 35% FC irrigated seedlings could be due to reduced availability and uptake of Mg from the soil or increased activities of chlorophyll catabolizing enzymes such as chlorophyll b reductase and Mg-dechelatase. In this case, irrigation at 35% FC did not significantly reduce available Mg in the soil (Table 2) but hindered its uptake by the root with the utmost consequence on chlorophyll biosynthesis, especially in the fast-growing semi-deciduous tree species.

To maintain similar growth attributes with the 80% FC-irrigated seedlings, the 60% and 35% FC-irrigated seedlings deployed both enzymatic and non-enzymatic antioxidant machinery. They (60% and 35% FC-irrigated seedlings) possibly activated ornithine aminotransferase and pyrroline-5-carboxylate reductase and deactivated proline oxidase and dehydrogenase to accumulate proline than the 80% FC-irrigated seedlings. Proline accumulation is the first response of plants to water/salinity-induced oxidative stress. According to Szabados and Savoure (2010), plants overcome stress-induced oxidative bursts by either increasing the activities of proline biosynthesis enzymes, specifically the ornithine aminotransferase and pyrroline-5-carboxylate reductase, or decreasing the activities of proline degradation enzymes specifically proline oxidase and dehydrogenase. Venekamp et al. (1989) suggested that plants increased de novo synthesis of proline from glutamate to overwhelm stress-induced active oxygen species. Similar results were reported for *Populus nigra* (Regier et al. 2009), *Conocarpus erectus* (Zafar et al. 2021b), *Acacia modesta*, and *Salix tetrasperma* (Rasheed et al. 2021) in water deficit conditions. At the same time, 60% FC and 35% FC-irrigated seedlings shielded their photosynthetic apparatus from photo-oxidation and maintained cellular membrane lipid integrity by activating phenolic-synthesizing enzymes - phenylalanine ammonia-lyase - and deactivating phenolic-catabolizing enzymes - polyphenol oxidase and peroxidase. This result is reflected in the higher total phenolic acid (TPC) content in the 60% and 35% FC-irrigated seedlings than in the 80% FC-irrigated seedlings. Phenolic compounds are non-enzymatic antioxidants (Khan et al. 2015) that neutralize free radicals (ROS) by quenching singlet and triplet oxygen and decompose peroxides like H2O2 in the plants' organelles (Osawa 1994). Similar results were reported for water-deficit-stressed *Brassica napus* (Akram et al. 2018) and *Syzygium cumini* (Zafar et al. 2021a). The lack of statistical difference between the ascorbic acid content of the 80% FC-irrigated seedlings and those of the 60% and 35% FC- irrigated seedlings suggests that these tropical trees species followed ascorbic acid pathway for combating oxidative stress and maintaining homeostasis in water stress conditions. Ascorbic acid is a powerful antioxidant that donates a hydrogen atom and forms a relatively stable ascorbyl-free radical in stress conditions. It plays a critical role in the photo-protection and synthesis of gibberellins and ethylene in woody plants (Chen et al. 2007).

Tree seedlings grown in the 60% and 35% FC irrigation regime also activated enzymatic antioxidant machinery by increasing the activities of SOD, CAT, and GPOx to surmount oxidative bursts. An increase in SOD, GPOx, and CAT activities in the 35% FC-irrigated seedlings and in CAT activity in the 60% FC-irrigated seedlings suggests the stepwise degradation of active oxygen species in the evaluated seedlings. In this regard, the tree seedlings activated the SOD enzyme first. Superoxide dismutase (SOD) is a known precursor of active oxygen derivatives such as peroxynitrite and hydroxyl radicals (Halliwell and Gutteridge 1999) and also produces H2O2 via dismutation of 02 in water stress conditions (Mittler 2002). However, the SOD-produced H2O2 is toxic to plant cellular organelles and disrupts several physiological processes in plants. Therefore, the evaluated tree seedlings subsequently activated CAT and GPOx enzymes which function in scavenging the SOD-synthesized H2O2. Second, exploration of the SOD, CAT, and GPOx by the 35% FC-irrigated seedlings and activation of only the CAT enzyme by the 60% FC-irrigated seedlings suggests a linear relationship between irrigation rates and enzyme antioxidants kinetics and type. In this case, 35% FC-irrigated seedlings increased the activities of both GPOX and CAT to scavenge overwhelming H2O2 (oxidative burst) produced in their cellular organelles (chloroplasts). In addition, 60% FC-irrigated seedlings needed to increase the activity of only CAT enzyme to survive mild oxidative bursts in their chloroplasts. However, the increase in SOD, GPOx, and CAT activities eventually paid off for the 35% FC-irrigated seedlings as it strengthened seedlings' tolerance by enhancing their water use efficiency (Figure 1) than seedlings in other regimes. Therefore, water deficit stress tolerance of the 35% FC-irrigated tree seedlings increased, a quality that will foster the post-transplant establishment and growth of the investigated tree species on the field. Similar results were reported for seedlings of *Morus alba* and *Conocarpus erectus* (Zafar et al. 2021a), *Acacia modesta* and *Salix tetrasperma* (Rasheed et al. 2021), *Prunus persica* (Haider et al. 2018) and *Olea europaea* (Ahmed et al. 2009).

Conclusively, the minimum irrigation rate for producing transplantable growth traits in the evaluated tropical tree species was 35% FC. The study recommends a 35% FC irrigation rate by nursery practitioners for turning out high-quality *T. catappa, B. monandra, and D. regia, V. merrillii,* and *D. lutescens* seedlings and saving about 31.7% of municipal water.

Interactive effects of water application rates and sawdust biochar on growth and biochemical constituents of five urban tree species used in urban landscaping in Ondo, Nigeria

The presence of biochar declined the water input and water use of the 35% FC-irrigated tree seedlings. However, only the water demand of 60% FC-irrigated seedlings was lower than that of the control seedlings at $P<0.05$. Interestingly, biochar enhanced the water use efficiency (WUE) of tree seedlings irrigated at 60% and 35% FC and slightly increased the WUE of the 80% FC-irrigated seedlings. Since the desired goal of every nursery operator is to produce quality, water-use-efficient tree seedlings, the application of biochar technology will therefore be beneficial for improving the quality of nursery trees and conserving municipal water resources while developing urban green infrastructure. Biochar addition in the soils consistently kept the leaf turgidity high, reduced the activities of SOD, CAT, and GPOx, and reduced the leaf proline, ascorbic acid, TPC, and TFC in the 80% FC-irrigated seedlings. Hence, biochars' performance was highest in the 80% FC-irrigated seedlings compared to those of the 60% and 35% FC-irrigated seedlings. A significant increase in growth attributes and biochemical constituents of

tree seedlings in the biochar regimes than those grown in the garden soil regime could be due to physical and chemical transformations induced by biochar in the soil-biochar mixture (sections 4.1 and 4.2). Chan et al. (2008) observed a significant increase in the yield of radish (*Raphanus sativus var.* Long Scarlet) when grown in a soil-biochar mixture and adduced their observation to the ability of biochar to increase available mineral nutrients and physicochemical properties of soil. Similarly, Hafeez et al. (2017) showed that transpiration and photosynthetic rates, water potential, and stomata conductance of biochar-treated Glycine max increased significantly and adduced it to the ability of biochar to increase soil moisture content and physicochemical properties.

Biochar-grown seedlings irrigated at 35% FC accumulated more proline, TFC, and TPC and showed higher kinetics of CAT enzymes which can aid the rapid establishment of transplants on the field. In addition, SH, LA, TLA, SLA, LAR, PGR, RGR, leaf RWC, chlorophyll content, carotenoid content, and activities of SOD and GPOx in biochar-grown seedlings irrigated at 35% FC was statistically similar to those in the control regime (that is, 80% FC-irrigated seedlings). This result implies that although both 80% FC and 35% FC irrigation techniques are viable for conserving water and nutrients inputs and improving WUE and tolerance of the tested seedlings to water stress, the performance of the 35% FC-irrigated seedlings was far better in the presence of biochar than in the garden soil without biochar application. Jaiswal et al. (2020) reported similar findings that the application of biochar strengthened both pathways and genes associated with plant defense in drought-stressed tomatoes. Khan et al. (2021) observed that biochar applied at the rate of 30 t ha-1 alleviated the negatives (effects) of drought stress in rapeseed and attributed it to 63, 48 and 62% increases in the activities of SOD, GPOx, and CAT respectively compared to stress-controlled rapeseed.

SUMMARY

The amount of water applied in the nursery significantly influenced the physical and chemical properties of the soil and the growth performance of five tree species grown on it. Soil bulk density (Bd) increased, but soil moisture content decreased at 35% and 60% FC irrigation rates. However, available K, Ca, and Mg and percentage N in the soil were slightly higher in the 80% FC irrigation rate than in the 60% and 35% FC irrigation rates. In the soil-biochar regime, Bd reduced significantly while porosity, moisture content, pH, organic matter content, available K, Ca, Mg, and P increased at 80% and 60% FC-irrigation rate relative to soil irrigated at 80% FC.

There were no significant differences between the growth attributes of the 80% FC-irrigated seedlings and those irrigated at 60% and 35% FC. Similarly, the malondialdehyde content of 80% FC-irrigated seedlings and those of the 60% and 35% FC-treated seedlings were statistically similar. Tree species grown in the 35% and 60% FC treatments deployed non-enzymatic and enzymatic antioxidant machinery to suppress stress and regulate oxidative bursts in the chloroplasts. Therefore, the minimum irrigation rate for producing transplantable growth traits in the evaluated tropical tree species was 35% FC. In soil-biochar mixture conditions, irrigation of tree seedlings at 80% FC produced the most vigorous and robust seedlings. However, irrigation of tree seedlings at a 35% FC rate showed attributes that could aid the rapid establishment of transplants on the field. The hypothesis of this study which states that biochar will improve soil physical and chemical properties and alleviate the adverse effect of water stress on the growth attributes of the five tree species' was confirmed.

CONCLUSION

This study reveals that irrigation of tree seedlings at 60% and 35% FC is a viable technique for producing quality tree seedlings while conserving substantial amounts of municipal water resources. The minimum irrigation rate for turning out high-quality seedlings of the evaluated tropical tree species was 35% FC. The study recommends a 35% FC irrigation rate for nursery practitioners to turn out high-quality *T. catappa, B. monandra, D. regia, V. merrillii,* and *D. lutescens* seedlings and save about 31.7% of municipal water. Biochar at all irrigation rates caused significant improvement in the soil physicochemical properties, moisture content, and water holding capacity. Biochar inclusion in the 35% FC-irrigated soil alleviated the adverse effects of water stress on growth attributes and strengthened the quality of the tree seedlings. In addition, biochar inclusion in the 80% and 60% FC-irrigated soil enhanced the trees' growth and visual quality at the seedlings stage.

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