Growth of *Moringa oleifera* Lam. as Affected by Biochar Treatment Modified *via* Inter-species Feedstock Selection

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To explore the impact of wood biochar on the early growth of tree seedlings, biochar was produced from the branches of tree species deemed as waste wood: tamarix, acacia, and eucalyptus. This biochar was mixed with agricultural soil at various concentrations. Subsequently, *Moringa oleifera* seedlings were planted in the biochar-soil mixture and monitored over an eight-week period. Then the data were collected and statistically analysed. All of the biochar treatments applied to *Moringa oleifera* seeds resulted in a notable reduction in germination rates. In particular, the control treatment—where no biochar was used—showed a significantly higher rate of seed germination compared to the various biochar treatments that were made from different feedstock species and processed at varying pyrolysis temperatures, highlighting the diverse impact of biochar characteristics on seed development. Nonetheless, the biochar-soil mixture retained higher levels of water and promoted greater biomass and relative plant growth. Thus, it is crucial to interpret these results within an environmental context to gain a comprehensive understanding. Selecting appropriate branch wood feedstocks may enhance the production of suitable biochar products for specific applications. Pretreatment techniques for feedstock before biochar processing might be necessary.

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Keywords: Growth traits; Moringa oleifera; Biochar characteristics; Feedstock selection; Tree species

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INTRODUCTION

Biochar has become a vital focus of research due to its numerous advantages and its significant potential in addressing pressing environmental and agricultural challenges. As a carbon-rich material produced through the pyrolysis of organic matter, biochar plays a critical role in improving soil health, enhancing carbon sequestration, and promoting environmental sustainability. However, the unique properties of biochar require careful consideration on regard to its optimal applications in various contexts. Researchers have highlighted two primary factors that are crucial in determining the characteristics and effectiveness of biochar: the type of feedstock used and the pyrolysis temperature (Lehmann 2007; Weber and Quicker 2018; Li *et al.* 2023).

The choice of feedstock, which can encompass a range of materials from agricultural residues to forest byproducts, directly impacts the nutrient content, surface area, and overall structure of the produced biochar. In contrast, the pyrolysis temperature affects the thermal decomposition process, influencing the stability, porosity, and capacity of biochar to adsorb pollutants and nutrients. Recent studies have underscored that both the selection of feedstock and the specific pyrolysis temperature are critical in shaping the physical and chemical properties of biochar. Understanding these aspects is essential for optimizing biochar production to achieve specific agricultural or environmental objectives, ultimately facilitating its effective use in promoting sustainable practices and enhancing soil quality (Lehmann 2007; Amin *et al.* 2016; Weber and Quicker 2018; Wahyuni *et al*. 2025).

The distinctive characteristics of biochar open up a range of exciting possibilities for enhancing its properties to suit specific applications. These properties, which can vary widely, can be strategically developed to meet the diverse needs of numerous sectors. A key factor in the production of biochar is the choice of feedstock, which significantly influences its final attributes. Exploring natural modification methods provides a promising avenue for optimizing these characteristics, enabling the creation of biochar with tailored functionalities that can effectively address challenges in various fields. A study conducted by Chemerys and Baltrėnaitė (2018) demonstrated that natural modifications are both cost-effective and readily accessible. However, their research did not address the extent to which these modifications influence biochar's efficacy when applied to soil or in environmental settings. It is worth noting that various studies have highlighted the distinctive behaviour of biochar in soil applications (Kammann *et al.* 2011; Eyles *et al.* 2015; Masiello *et al.* 2015; Aller 2017; Liu *et al.* 2017).

The role of biochar characteristics, which are shaped by the choice of feedstock species, on plant growth remains a relatively unexplored area of research (Zhang *et al.* 2017; Ippolito *et al.* 2020; Li *et al.* 2023). Biochar is known for its potential to improve soil health and enhance plant performance; however, research on how different feedstock sources affect biochar properties and plant growth is limited. This study hypothesizes that the properties of biochar—such as nutrient content, pH, surface area, and porosity—are significantly influenced by the type of feedstock used. Different feedstock materials yield biochar with unique characteristics that affect their effectiveness in soil amendment and plant growth. Therefore, selecting the appropriate feedstock is crucial for ensuring biochar is suitable for specific agricultural and environmental goals. This choice also allows for the customization of biochar properties to match local soil conditions and plant needs, making it essential for sustainable agriculture and climate mitigation (Novak *et al.* 2009; Singh *et al.* 2010; Lehmann and Joseph 2015).

The research focuses on the effects of biochar from various feedstock species on the germination and early growth of *Moringa oleifera*. A plant known as the "miracle tree", valued for its nutritional and medicinal benefits, is increasingly seen as a strategic plant in the Middle East in response to climate change adaptation, land degradation, and the greening goals of many Arab countries (Cao *et al.* 2023). Initiatives include Saudi Arabia's Green Initiative and the UAE's "Greening the Desert" plan to plant over 50 billion trees in the region, requiring resilient species such as *Moringa* sp. Although *Moringa oleifera* is drought-tolerant and grows well in poor soils, its cultivation in arid areas, particularly in the Middle East, faces significant challenges. These can be categorised into biophysical, environmental, socio-economic, and technical constraints (Arif *et al.* 2020; Farooq *et al.* 2022). Through examining how the distinct traits of biochar impact growth metrics, this study aims to optimize biochar applications for better growth performances and identify the most effective feedstock inter-species selection from a specific part of the tree for enhancing *M. oleifera* cultivation. While whole stem wood and agricultural residues have been widely studied, branch wood between inter-species is relatively underexplored and possesses unique anatomical and chemical properties that can influence the quality of the resulting biochar. However, research in this area remains limited.

EXPERIMENTAL

Materials

The wood feedstocks from three different tree species selected in this study were (1) grey-haired acacia (*Acacia gerrardii*); (2) athel tamarisk (*Tamarix aphylla*); and (3) red gum (*Eucalyptus camaldulensis*). These species were selected as feedstocks due to their availability in the arid region and represent great variability among their traits (Suansa and Al-Mefarrej 2020). Three trees for each species and three branches from each sample tree were cut just 20 cm above the basal collar/swelling to avoid any variability (Zhao *et al.* 2019). All samples were taken from the Agricultural Research and Experiment Station, Dirab, South of Riyadh (24º 24' 31.93" N, 046º 39' 41.16" E; 584 above sea level). The area has a Mediterranean climate with annual maximum and minimum temperatures of about 45 and 5 °C, respectively. The monthly precipitation is a maximum of 30 mm. The soil pH is slightly alkaline at about 7.5 (Mefarrej 2001). *Moringa oleifera* was selected as a target species because of its sensitivity to assess the climate environmental change in the early stage of seedling development (Moura *et al.* 2021), commercially important food, and fast growth rate in response to water (Noulèkoun *et al.* 2018).

Methods

Biochar production

Biochars were made by slow pyrolysis under oxygen-limited circumstances. The chips of feedstock were air-dried for 7 days after cleaning with distilled water, oven-dried overnight at 103 ± 2 ºC, and tightly packed into an iron container. Then it was charred in a muffle furnace under oxygen-limited conditions, heated at a rate of 8 to 10 ºC min^{-1}, and then kept at the highest pyrolysis temperature for 1 h under atmospheric pressure. The pyrolysis temperatures of 300, 400, and 500 ºC were selected. The biochar was left in the muffle furnace overnight at the end of the production process. Biochar was mixed with distilled water on an orbital shaker table rotating at 120 rpm for 24 h to obtain solutions with a final concentration of 2% (v v^{-1}). The slurries were suction filtered, and the solution was collected for further stages.

Seed germination

The germination test was performed in 9-cm disposable Petri dishes, each containing a filter paper, with 5 mL of distilled water (control) or 5 mL of each treatment solution (biochar leachate with concentration 2% v v^{-1}) added to each replicate. Seven seeds of *Moringa oleifera* were placed on each Petri dish for each treatment. Those Petri dishes were watered periodically to keep moist with 1 mL of each treatment solution or 1 mL of distilled water (control). The Petri dishes were kept at room temperature for 10 days. Seeds were considered germinated when the radicle had emerged 1 mm from the seed coat (Dadkhah 2013; Júnior *et al.* 2025). The final germination percentage was calculated according to Agrawal (2011). The germination speed was calculated using the coefficient of the velocity of germination (CVG) according to Nichols and Heydecker (1968). Concerning early development, lengths (mm) of radicle-hypocotyl were measured for 3 individual seeds in each Petri dish. The vigor index (VI) of early growth development was estimated as suggested by Abdul-Baki and Anderson (1973).

Seedling experiment

The seedling experiment was conducted under a non-controlled greenhouse that has average day and night temperatures of about 37 ºC and 28 ºC, respectively. Biochar was incorporated at a rate of 2% (w w^{-1}) or approximately 44 tons ha^{-1} for all treatments. Each plastic pot (n = 48 pots) of 9 cm diameter and 10 cm depth was filled with 0.5 kg of sterilized sandy soil (for control) or 0.5 kg of biochar-sterilized sandy soil mixture (for treatments) and manually tapped down to consolidate the soil. Thereafter, two seedlings of *M. oleifera* (10 days after sowing) were transplanted to each pot and watered periodically (50 mL per day). Plants were measured weekly to obtain several data involving the relative growth rate of seedling height (RGR_H), the relative growth rate of seedling diameter (1 cm from the soil surface) (RGR_D), and the relative growth rate of the number of leaves (RGR_L). Once seedlings reached the 3rd week after transplanting, plants were thinned to one plant per pot (the largest plants were kept). The pots were watered once and subsequently no watering was applied. Moisture content and weight of seedling biomass were determined at the end of the experiment (until the plant death by drought stress).

Experimental design and statistical analysis

These experiments were performed to reveal the effect of tree species (feedstock) and pyrolysis temperatures. Therefore, a two-factorial experiment was conducted in a randomized completely block design (RCBD). The first factor was tree species, which involved three species (*A. gerrardii*, *T. aphylla*, and *E. camaldulensis*). The second factor was pyrolysis temperature, which involved three levels (300, 400, and 500 ºC). It involved 9 different treatments with four replicates. Hence, the total of the experimental unit is 36 units for biochar characterizations and 48 units for the seedling experiment, where 36 samples are the biochar-soil mixture, and 12 samples are soil without biochar as control. All data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS) software (SAS, ver 9.2, SAS Institute Inc. Cary, NC, USA). The Least Significant Difference (LSD) test at 0.05 level will be used to compare the significant difference among the means.

RESULTS AND DISCUSSION

Biochar Effect on Germination of *Moringa oleifera*

All the biochar treatments in this study suppressed the germination of *M. oleifera* seeds. Table 1 shows that the control treatment was significantly higher compared either to the feedstock species or pyrolysis temperature ($p < 0.05$). Approximately 79.7% of *M. oleifera* seeds germinated under control treatment. Moreover, seed germination under control treatment was higher than any biochar product originated from *A. gerrardii* (53.6%), *T. aphylla* (54.8%), and *E. camaldulensis* (57.1%). The CVG of the control treatment was about 38.9%. It was higher than *A. gerrardii*, *T. aphylla*, and *E. camaldulensis*, which had CVG of approximately 23.5%, 22.2%, and 26.1%, respectively. Furthermore, the VI of the control treatment was approximately 2,847.9, and it was higher than *A. gerrardii*, *T. aphylla*, and *E. camaldulensis*, which had VI values of approximately 1,554.9, 1,569.5, and 1,814.8, respectively.

The control treatment was markedly higher compared to the other biochar treatments that originated from either different feedstock species or pyrolysis temperatures. There was no different effect among the different feedstocks; in the present study, the leachate from fresh biochar is highly potentially toxic to seed germination. Intani *et al*. (2019) also found that water extracted from fresh biochar showed acute and severe toxic effects on the germination of cress seed, and biochar administration can slow down germination if given in very high doses (> 40 to 50 tons ha^{-1}). Several compounds have been reported to be responsible for the biochar potential toxic effects on seed germination, such as fatty acids (Shiralipour *et al.* 1997; Navajas-Porras *et al.* 2024), heavy metals (Li *et al.* 2005), volatile organic compounds (VOCs) (Freddo *et al.* 2012), polycyclic aromatic hydrocarbons (PAHs) (Rogovska *et al.* 2012), ammonia (Qi *et al.* 2012), and salts (Panuccio *et al.* 2014).

Table 1. Effect of Biochar on Germination Percentage (GP), Coefficient of the Velocity of Germination (CVG), and Vigor Index (VI)

Species	Temperature (°C)	GP (%)	CVG (%)	VI
A. gerrardii	300	32.1 ± 6.84^{c}	25.4 ± 1.15^{b}	886.3 ± 163.71^{c}
	400	71.4 ± 8.25^{b}	20.0 ± 2.15^{b}	1,912.4 ± 362.56^{b}
	500	57.1 ± 13.04^{ab}	25.1 ± 1.08^{b}	1,866.2 ± 549.52^{b}
T. aphylla	300	42.9 ± 14.29^{c}	25.0 ± 1.46^{b}	1,204.6 ± 285.5^{c}
	400	46.4 ± 6.84^{b}	20.8 ± 2.59^{b}	1,180.6 ± 169.97^{b}
	500	75.0 ± 6.84^{ab}	20.8 ± 1.29^{b}	2,323.2 ± 287.46^{b}
E. camaldulensis	300	35.7 ± 12.37^{c}	28.3 ± 7.38^{b}	1,056.8 ± 364.14^{c}
	400	64.3 ± 17.00^{b}	24.6 ± 1.6^{b}	2,417.2 ± 762.03^{b}
	500	71.4 ± 8.25^{ab}	25.5 ± 3.78^{b}	1,970.4 ± 258.31^{b}
Control		79.7 ± 4.11$^{A(a)}$	38.9 ± 3.46$^{A(a)}$	2,847.9 ± 180.91$^{A(a)}$

Regarding the pyrolysis treatment, the GP, CVG, and VI were significantly less at the lower temperature when compared to the higher temperature. The GP of the control treatment was higher than any biochar product originating from the pyrolysis temperature of 300, 400, and 500 °C, about 53.7%, 23.8%, and 14.9%, respectively. The CVG of the control treatment was higher than that produced biochar of pyrolysis temperatures at 300 °C, 400 °C, and 500 °C, about 32.7%, 44.0%, and 38.9%, respectively. The VI of the control treatment was higher than that produced biochar of pyrolysis temperatures at 300 °C, 400 °C, and 500 °C, about 63.2%, 35.5%, and 27.9%, respectively.

Results showed that the higher the pyrolysis temperature, the lower the toxic effect of biochar leachate. In agreement with the present study, Lehmann (2007) stated that pyrolysis temperature is the foremost factor that determines the biochar characteristics mentioned above, the temperature of 300 °C is not sufficient to break down PAHs and VOCs. At the lower temperature, these compounds re-condense onto biochar. Some studies observed that the remaining PAHs and VOCs become decomposed at a temperature between 400 and 500 °C (Weber and Quicker 2018) or above 500 °C (Kinney *et al.* 2012; Gray *et al.* 2014; Das and Sarmah 2015). Hence, the potential toxic effect of biochar decreases up to sufficient temperature to break down those compounds. Post-treatment procedures might be important to diminish the potential toxicity of VOCs (Gale *et al.* 2016). The potential toxic effects of fresh biochar may be decreased in simple ways such as washing (Busch *et al.* 2012), drying treatment (Koltowski and Oleszczuk 2015), and purging gases in the production process (Intani *et al.* 2019).

Table 2. Effect of Biochar Produced on Plant Growth Conditions During Drought

Species	Temperature (°C)	Day 1	Day 2	Day 3	Day 4	Day 5
A. gerrardii	300					
	400					
	500					
T. aphylla	300					
	400					
	500					
E. camaldulensis	300					
	400					
	500					
Control						
Colour Indicator	**Plant Condition**					
	Health up to 80% in good condition					
	25% wilting					
	50% wilting					
	75% wilting					
	Plant death					

Biochar effect on early seedling growth of Moringa oleifera

The conditions of plant growth during the drought period significantly declined, particularly under the control treatment (Table 2). The lower drought stress effect was exhibited by *T. aphylla*, followed by *A. gerrardii* and *E. camaldulensis*. Under control treatment, the seedlings wilted on the second day of the drought period and were completely dead on the fifth day. Amplifying biochar to the soil increases plant-available water, which can be attributed to greater water held at field capacity (Gaskin *et al*. 2007; Hansen *et al*. 2016; Aller *et al*. 2017). It improves the soil properties by increasing the proportion of large voids and air-filled porosity and by reducing the bulk density of the soil (Jones *et al.* 2011). However, this effect is likely dependent on the types of soil. Masiello *et al*. (2015) stated that the hydrological effect of biochar could not be entirely characterized before being added to the soil. Hereafter, Aller *et al*. (2017) reported that the effect of biochar on soil moisture increased in sandy soil, decreased in clayey soil, and had no impact in loamy soil.

Figure 1 shows that seedling moisture content (MC) of *M. oleifera* under control treatment during the drought period was significantly lower either compared to the feedstock species or pyrolysis temperature ($p < 0.05$). Compared to the control, biochar produced from *T. aphylla*, *A. gerrardii*, and *E. camaldulensis* generated higher MC in the seedlings of *M. oleifera* about 152.7%, 139.9%, and 81.7%, respectively. By contrast, the MC of *M. oleifera* was significantly less in the produced biochar of pyrolysis temperature at 500 °C when compared to 300 °C. Control treatment differed from produced biochar of pyrolysis temperatures at 300, 400, and 500 °C about 146.4%, 116.2%, and 111.7%, respectively. Additionally, the MC in the root of the seedling was higher when compared to the shoot and leaves. The biochar originating from *T. aphylla* supported the early seedling growth more than the other feedstocks. This may occur because the parent material had the highest porous structure and mineral content (salts) (Newete *et al.* 2019). Masiello *et al*. (2015) explained that even though salts do not directly affect the WHC, these substances could change the amount and composition of salts in the soils. Subsequently, they alter the soil water potential.

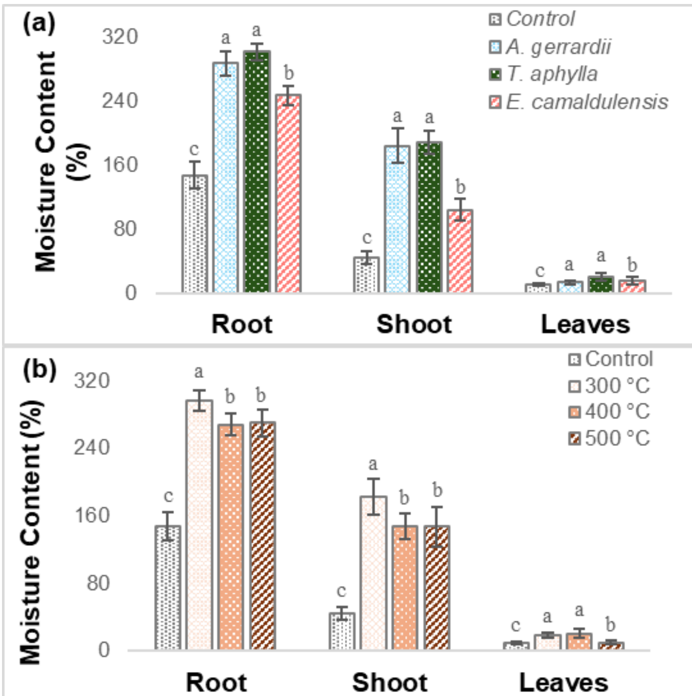

Fig. 1. Moisture content of seedlings in the root, shoot, and leaves based on (a) feedstock and (b) pyrolysis temperature; Different letters in each part correspond to significantly different values ($p < 0.05$)

The relative growth rate of height (RGR_H), diameter (RGR_D), and the number of leaves (RGR_L) in the control treatment were a lot less than the other treatments (Fig. 2). The addition of biochar to the soil resulted in greater growth in seedlings of *M. oleifera*. However, the effects varied depending on the feedstock and pyrolysis temperature. The highest RGR_H and RGR_D values generated by biochar originated from *T. aphylla.* The averages were higher by about 179.8% and 109.6%, respectively, when compared to the control. The highest RGR_L generated by biochar originated from *E. camaldulensis*. The value was higher by about 47.4% when compared to the control. Furthermore, a higher pyrolysis temperature resulted in a higher RGR_H value. In contrast, a higher pyrolysis temperature resulted in a lower RGR_D value. However, there was no significant difference among pyrolysis temperature on RGR_L ($p < 0.05$).

The higher relative growth rate (RGR) of *M. oleifera* was attained under the *T. aphylla* treatments. This was possibly due to an effect of the high extractive and ash content in the feedstock and the higher EC in the biochar product or the percentage of the vessel cell lumen (Suansa *et al.* 2021). Amonette and Joseph (2009) found that the persistence of oxygen functional groups in extractives during the pyrolysis process contributes to a higher cation exchange capacity (CEC). Subsequently, this impacts the ability of biochar to retain some nutrients available to target species (Veiga *et al.* 2017). Jin *et al*. (2016) mentioned that biochar could be a source of nutrients. However, Tammeorg *et al*. (2017) reviewed nutrient content of biochar to be low. Glaser *et al*. (2002) gave an exception in that nutrient-rich feedstock generated ash-rich biochar. Moreover, Kloss *et al*. (2012) and Nanda *et al*. (2013) found that biochar with higher ash, nutrient content, EC, and thermal stability generally offers higher amounts of nutrients, and thus potential as a soil conditioner. Hence, the ash content during the conversion of biomass into biochar may become a good indicator to determine the suitability of biochar application for increasing soil fertility.

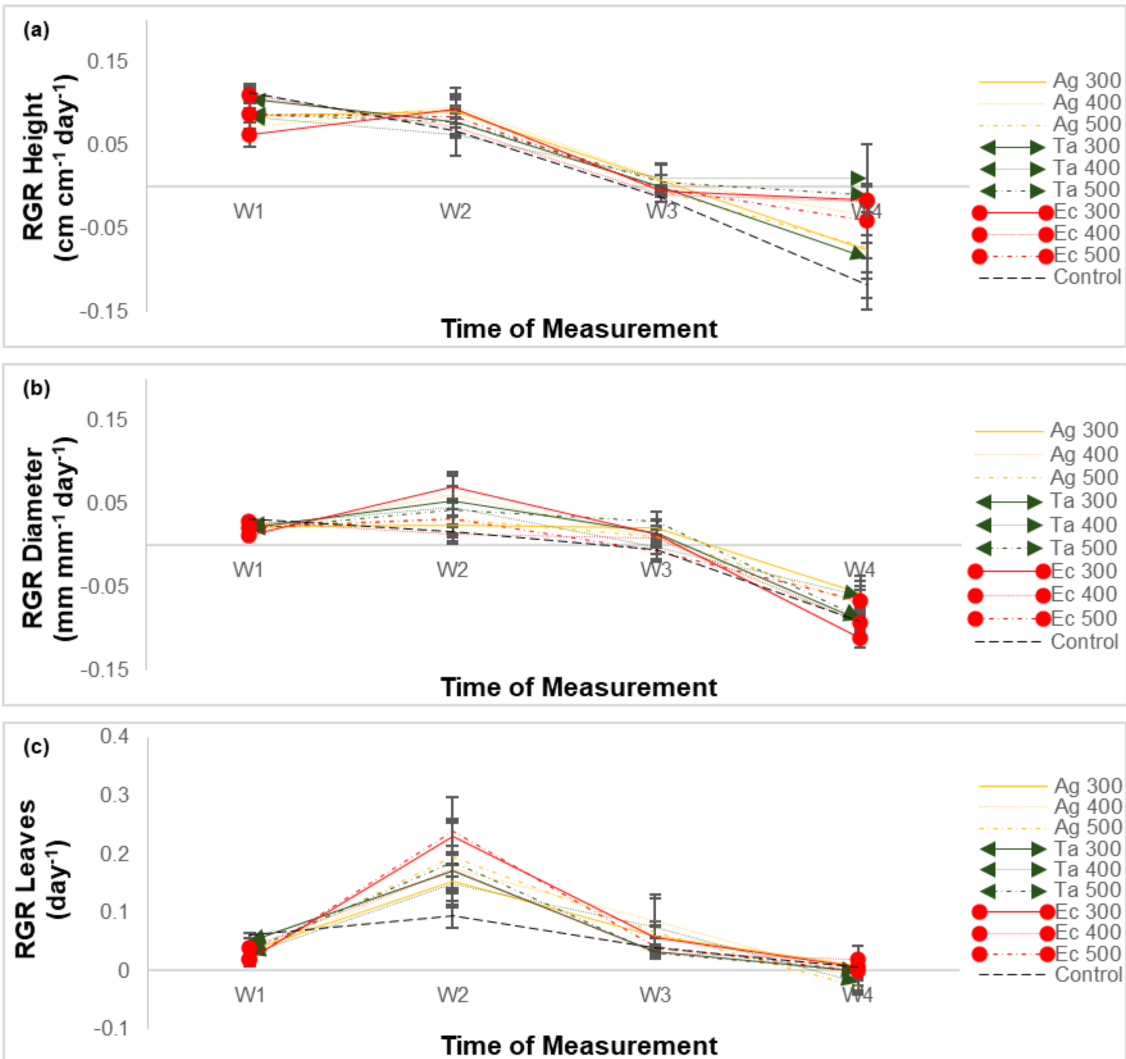

Fig. 2. The effect of biochar produced by different pyrolysis temperatures on the relative growth rate of *M. oleifera* seedling: (a) height, (b) diameter, and (c) leaves (mean ± SE); A symbol W1 up to W4 indicates the time of measurement from 1st week up to 4th week

The biomass of roots and leaves in the seedlings was highly affected by biochar originating from *E. camaldulensis*, followed by *A. gerrardii* and *T. aphylla*. Figure 3a shows that compared to the control, biochar originating from *E. camaldulensis*, *A. gerrardii*, and *T. aphylla* increased the root biomass approximately 46.8%, 28.6%, and 5.2%, respectively, and increased the leaves biomass approximately 30.4%, 14.0%, and 11.5%, respectively. Moreover, the biomass of root was significantly less in produced biochar of pyrolysis temperature at 300 °C when compared to 500 °C. Figure 3b shows that compared to the control, biochar originated from the pyrolysis temperatures of 300, 400, and 500 °C increased the root biomass approximately 17.1%, 33.4%, and 30.1%, respectively.

Results revealed that an increase in plant growth was not always associated with an increase in biomass yield. The various findings are also exhibited by several studies. Aller *et al*. (2017) found that biomass weight increased with the addition of biochar in the sandy and loamy soil and decreased in the clayey soil compared to the control. The authors also reported that there was no significant effect of adding biochar on plant height. Hansen *et al*. (2016) found that the addition of biochar to sandy loam soil does not increase shoot growth. This variation may be explained by several factors, such as responses of target plant to biochar treatments, depth and method of biochar incorporation, the difference in biochar properties, and biochar-soil interaction (Zobel and Van Buijtenen 1989; Kammann *et al.* 2011; Eyles *et al.* 2015).

The effects of growth rate and nutrients cannot be separated because increased growth is usually a result of nutrient availability (Lambers *et al.* 1998). Accordingly, Zobel and Van Buijtenen (1989) reported that nutrient deficiencies cause a high wood SG, while added fertilizers and increased nutrient availability might influence decreasing the SG, cell size, and wall thickness for further reduced biomass on plants under fertile conditions. However, Payen *et al*. (2016) explained that an increase in soil EC corresponds to an increase in salinity derived from saline biochar may lead to soil infertility. Therefore, careful land management should consider the type of soil on which the biochar is applied. As an example, maintaining the irrigation and drainage avoids the build-up of ions in the root zone, thus reducing the exposition of plants to various potential stress effects (Payen *et al.* 2016; Marmiroli *et al.* 2018; Intani *et al.* 2019). While pretreatment techniques for feedstocks prior to biochar processing may be beneficial, this area is still underexplored and warrants further investigation. To enhance our understanding, it is imperative that additional research be conducted. This study presents compelling evidence indicating that certain chemical compounds exhibit toxicity, particularly within the filtrates of lower-temperature biochar. Future investigations should focus on exploring the relationships between variables such as pH and metal concentration, as these may act as significant controlling factors. Moreover, due to the unique characteristics of biochar-soil mixtures, conducting long-term studies under field conditions is essential for gaining deeper insights and refining future applications.

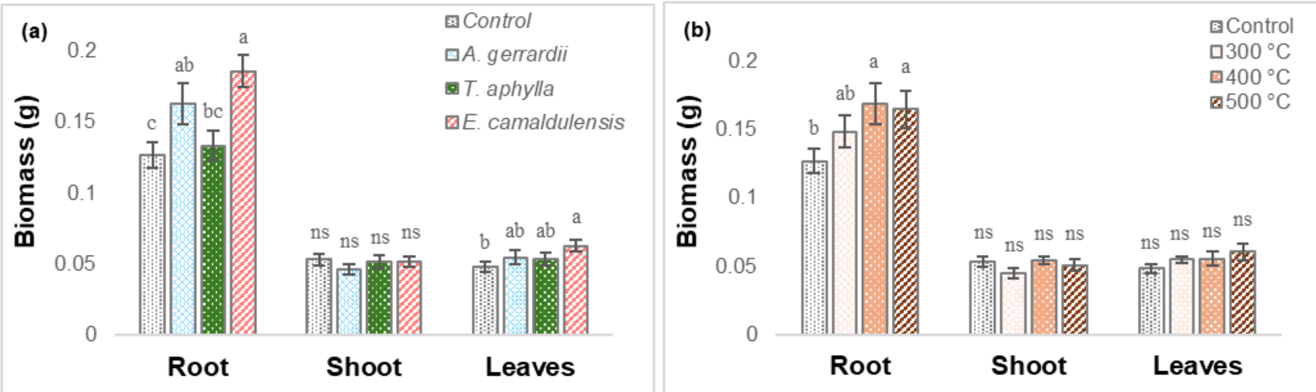

Fig. 3. Biomass allocation of seedlings in the root, shoot, and leaves based on (a) feedstock and (b) pyrolysis temperature; Different letters in each part correspond to significantly different values ($p < 0.05$)

CONCLUSIONS

1. Each biochar treatment examined in this study effectively suppressed the germination of *Moringa oleifera* seeds.
2. Raising the pyrolysis temperature significantly reduces the toxic effects of biochar leachate, making it a safer alternative for environmental applications.
3. The biochar-soil mixture showed better water retention, boosting biomass and plant growth. The least drought stress effect was observed with biochar derived from *Tamarix aphylla*, followed by *Acacia gerrardii* and *Eucalyptus camaldulensis*.

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