



## Biomass Production and Partitioning Response of Coffee (*Arabica Coffea L.*) Seedlings to Different Poly-Bag Size and Biochar Based Media Preparations

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### Article History

Received: 07.04.2024

Accepted: 15.05.2024

Published: 23.05.2024

**Abstract:** Nursery experiment was conducted at Awada Agricultural Research Sub-Center, to investigate dry matter production and its partitioning into above and below ground parts of Arabica coffee seedlings treated under different ratio of biochar to top soil based media preparations and different poly bag size used to raise the seedlings. The treatment combinations with four levels of pot size (width by height) (7cm x13cm, 10cm x 16cm, 13cmx19cm and 16cmx 22cm) and five levels of biochar to top soil ratio (0:1, 1:1, 1:2, 1:3, 1:4) by (v/v) were used for the treatment were factorially combined and laid out in a randomized complete block design with three replications. Results showed that the main effect of poly bag size and biochar to top soil ratio of media preparation were significantly ( $P<0.01$ ) affect leaf, shoot, root and total dry matter production and shoot to root ratio of coffee seedlings. The highest total dry weight (6.83g) was recorded from pot size of 16x22cm interacted with one to three (1:3) biochar to topsoil ratio followed by (6.57g) harvested from pot size of 13cmx19cm interacted with one to four (1:4) biochar to top soil ratio. Similarly, the root to shoot ratio of coffee seedlings was significantly ( $P = 0.017$ ) affected by the combined effects of the two factors. Pot size 13cmx19cm interacted with one to four (1:4) biochar to top soil ratio exhibited the maximum (0.272g/g) RSR as compared to rest of growing media and all pot size interacted with one to four (1:4) biochar to top soil ratio have statically higher value similar to the largest value above mentioned parameters except shoot to root ratio. Thus, it is concluded that vigorous coffee seedlings with high dry matter content and optimum root to shoot distribution for better seedling production can be produced by optimum poly bag size of 13cmx19cm interacted with one to three (1:3) biochar to topsoil. But, further investigations should be continued under different media preparation for wider opportunities at different area coffee growing areas.

**Keywords:** Arabica Coffee, Biochar, Dry Matter, Partitioning, Seedling, Poly Bag.

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## 1. INTRODUCTION

Commercially coffee is propagated through seeds with a various factors that influence the initial development of coffee seedling and/or growing in the field, such as the seedlings production process specially, container size and the substrate and seed quality used to raise coffee seedling are driving

factors for quality seedling production. Container size determination such as length, height, volume and shape affect the size depth and volumes of the root system and also its distribution based on, availability and absorption of nutrient and water in the substrate (Salisu, *et al.*, 2018). Root growth usually occupies the space of its container and optimal root growth and distribution depends on the physical rooting

**Citation:** Leta Ajema Gebisa (2024). Biomass Production and Partitioning Response of Coffee (*Arabica Coffea L.*) Seedlings to Different Poly-Bag Size and Biochar Based Media Preparations, Glob Acad J Agri Biosci; Vol-6, Iss- 3 pp- 48-56.

environment which is the container size. It has been proved that a larger container size increased root mass in plant. The inadequate container size usually causes root restriction and decrease both shoot and root dry matter and total leaf area. Under ample supply of water and nutrient, root growth is the major factor that controls the shoot growth (Zakaria, *et al.*, 2021). In the nursery, the use of appropriate growing media is critical for the development of high-quality seedlings because it has a direct impact on the growth, development, and maintenance of a healthy root system. Topsoil is a typical growing media used in nurseries to raise seedlings (Marjenah, *et al.*, 2016) as well as a factual for coffee seedling production in Ethiopia. However, there is a need to maintain the fertility status of the topsoil used to raise coffee seedlings in the nursery affected by the degradation of land caused by the ever-increasing demand to enlarge agricultural areas with economically and space optimum poly bag sizes for wider production opportunities. Therefore, this experiment was designed to determine the optimum poly bag size with appropriate biochar based nursery media preparation that enhance the best combination of shoot to root dry matter partitioning and enhance the seedling growth.

## 2. MATERIALS AND METHODS

### 2.1. Description of the Study Area

The study was conducted at Awada Agricultural Research Sub-Center (AARSC). It is 315 km far away from Addis Abeba close to Yirgalem town situated in the moderate to cool semi-arid mid highland agro-ecology of south Ethiopia (Alemayo D, 2019) that located at 6°3'N Latitude and 38°E Longitude at an altitude at an elevation of about 1740 meter above sea level. The area has a semi-bimodal rainfall distribution characterized by double wet and dry seasons with an average precipitation of 1342 mm per annum, while the annual average minimum and maximum air temperatures are 11°C and 28.4°C, respectively. The major soil types are *Nitisol* and chromatic-cambisols that are highly suitable for coffee production (Ajema *et al.*, 2022).

### 2.2. Experimental Material

A released coffee variety Koti (85257) which is an open canopy type and recommended for the study areas was used to experiments. Biochar was prepared from coffee husk collected from the dry pulping station found near to AARSC. The collected husk was air dried in open sun. Two different (small and large) sized barrels were used to produce biochar. The dried husk was filled in to the smaller barrel. The barrel filled with husk was inverted in the large barrel to minimize the oxygen and facilitate the pyrolysis process. The resulting biochar material was grounded and sieved through 2mm square-mesh sieve to have the same particle sizes with topsoil used

for experiment. The dark color pots with different width of (7cm, 10cm, 13cm, and 16cm) with the adjusted length for each treatment were used for the experiments. Topsoil was collected from the surface of cultivable lands at 0-15cm depth, dried and crushed by pestle and mortar and sieved with two mm square-mesh sieve to have the smaller particle size. Compost was also prepared from locally available materials. The nursery media adopted by farmers in the study area was prepared by mixing topsoil and compost in three to one (3:1) ratio as stated by (Antenehe *et al.*, 2015). The media was thoroughly mixed with biochar following the adjusted ratio for each treatment.

### 2.3. Experimental Design and Treatment Combinations

The experiment was conducted with factorial experiment arranged in a randomized complete block design (RCBD) with three replications to provide estimates of treatment effects or differences among treatment effects. Twenty four (24) treatment combinations with four levels of pot size (width by height) ( $P_1 = 7\text{cm} \times 13\text{cm}$ ,  $P_2 = 10\text{cm} \times 16\text{cm}$ ,  $P_3 = 13\text{cm} \times 19\text{cm}$  and  $P_4 = 16\text{cm} \times 22\text{cm}$ ) and five levels of biochar to top soil ratio (0:1, 1:1, 1:2, 1:3, 1:4) by (v/v) were used for the treatment. The conventional pot (16x22 cm) filled with Top soil +2gDAP was used as a positive control, while topsoil alone in the same size (16x22cm) was used as a local control.

### 2.4. Data to Be Collected

The destructive and non destructive data were recorded at the end of the experiment in August 2018. Coffee seedling growth parameters were recorded using appropriate measurement materials. The destructive data were also recorded to evaluate growth of each plant part. Shoot and root parts of the seedlings were separated by cutting plant at the collar point using scissors. Then, shoot parts were separated in to leaves and stems, and their fresh weights were measured by sensitive balance and expressed in gram per plant. All plant parts were separately placed in labeled paper bag and dried in oven dry at 70°C until the constant weight and dry matter yield was measured for each sample using sensitive balance. The dry weight of each plant part, was used to determine the dry matter partitioned to stem, leaves and roots and total dry matter yield (stem + leaves + roots dry matter) of the seedlings (Yacob *et al.*, 1995). Then the following parameters were calculated from the already indicated measured plant parts.

- 1) Shoot mass ratio (SMR=shoot dry weight/total dry matter)  

$$\text{SMR} = \text{SDW}/\text{TDW}$$

- 2) Root mass ratio (RMR = Root dry weight/total dry matter)  
RMR = RDW/TDW
- 3) Root to shoot ratio (RSR = root dry weight/shoot dry weight)  
RSR = RDW/SDW

### 3. RESULT AND DISCUSSIONS

#### 3.1. Dry Biomass Weight of Coffee Seedling

##### 3.1.1. Leaf Dry Weight

A different poly bag sized and biochar applications ratios significantly (P=0.001) affected leaf dry weight of coffee seedlings (Appendix Table 1). The maximum production of leaf dry weight of coffee seedlings (4.17g) was recorded for 13x19cm pot size followed by (4.12g) from the largest (16x22cm) pot size filled with one to three biochar to topsoil ratio, while the lowest values (2.88g) weight was recorded for the smallest pot size without biochar (Table 1). This might be due to favorable growth conditions for better leaf growth of the seedling, particularly for good development of the root system to access available nitrogen in the media at the optimum ratio of a larger container size.

Addition of biochar into soils as a conditioner enhance bioavailability of nitrogen, phosphorus and Potassium there by plant growth at most relatively lower application rate. The lower leaf dry weight in high amount of biochar to topsoil ratio might be due to the antagonistic effects of biochar to nutrient availability at higher ratio of application. It has been reported that, biochar addition did not always increase soil plant nutrient, especially phosphorus availability at higher application rate (Rangaswami, 2018). Novak *et al.*, (2009), have also reported that biochar application significantly increased phosphorus retention in soils and decreased phosphorus levels in leachate solutions in a soil column experiment. The experiments conducted by Al-Wabel *et al.*, (2018) have shown that, biochar enhanced lettuce growth by increasing the dry biomass by 114%). Biochar application showed higher dry matters of leaf, stem, and total biomass production compared with no biochar application. Gebremedhin *et al.*, (2015) have reported the significantly increased grain and straw yields of wheat by 15.7% and 16.5% respectively, over the NP application (control).

**Table 1: Leaf dry weight (g) as affected by biochar ratio and pot size interaction**

Biochar ratio						
Pot size(cm)	Top Soil	1BC:1TS	1BC :2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	2.88 <sup>m</sup>	3.12 <sup>iljk</sup>	3.20 <sup>ghij</sup>	3.36 <sup>fgh</sup>	3.40 <sup>efg</sup>	3.21 <sup>ghij</sup>
10x16	2.93 <sup>lm</sup>	3.20 <sup>hijk</sup>	3.22 <sup>ghij</sup>	3.62 <sup>de</sup>	3.42 <sup>efg</sup>	3.39 <sup>efgh</sup>
13x19	2.95 <sup>klm</sup>	3.10 <sup>jklm</sup>	3.46 <sup>ef</sup>	4.17 <sup>a</sup>	3.89 <sup>bc</sup>	3.85 <sup>c</sup>
16x22	3.00 <sup>jklm</sup>	3.04 <sup>jklm</sup>	3.34 <sup>fghi</sup>	4.12 <sup>ab</sup>	4.02 <sup>abc</sup>	3.83 <sup>cd</sup>
CV (%)		4.14				
LSD (0.05)		0.23				

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05.

##### 3.1.2. Shoot Dry Weight

The shoot dry weight of the seedling was significantly (P=0.0003) influenced by different containers (Appendix Table 1). The maximum shoot dry weight of coffee seedlings (5.41g) followed by (5.40g) were recorded from the interaction effects of the largest or control 16x22cm pot size with one to three and one to four ratio of biochar to topsoil respectively. The conventional pot size interacted with one to three ratio of biochar increased the seedling shoot dry weight by 34% over the local practice. This might be due to combination of a good biochar based growing media along with good container, which leads to increase biomass or dry weight of seedlings. On the other hand, the minimum shoot dry weights of (3.76g) and (3.94g) were recorded from the interaction effects of 7x13cm and 10x16cm pot size without any biochar or inorganic

fertilizer amendments respectively (Table 2). Biochar improved soil fertility by increasing some plant nutrients that could resulted in increased shoot dry biomass due to the uptake of these nutrients by coffee seedling. The contents of N, P and K, were increased with the lower rate of biochar application (Ajema *et al.*, 2022). This result in line with study of Majeed *et al.*, (2018) who reported that availability of Ca, K, P, Cu, and Zn increased with the biochar applications especially at 4% dose. According to Liang *et al.*, (2014) reported that an increased nitrogen availability with biochar applications increased total nitrogen and nitrate concentrations but caused a decrease in ammonium levels of soils. The most important drawback of biochar applications found in this study was the effects of high pH biochar on soil pH. Slightly alkaline soil pH values (7.43) increases might affect availability of plant nutrients.

**Table 2: Shoot dry weight (g) as affected by biochar ratio and pot size interaction**

Biochar ratio						
Pot size(cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	3.76 <sup>j</sup>	4.07 <sup>ghi</sup>	4.21 <sup>fgh</sup>	4.50 <sup>efd</sup>	4.64 <sup>ed</sup>	4.41 <sup>edf</sup>
10x16	3.94 <sup>hij</sup>	4.10 <sup>ghi</sup>	4.22 <sup>fgh</sup>	4.70 <sup>d</sup>	4.63 <sup>ed</sup>	4.63 <sup>ed</sup>
13x19	4.06 <sup>hi</sup>	3.91 <sup>ij</sup>	4.40 <sup>ef</sup>	5.16 <sup>abc</sup>	5.16 <sup>abc</sup>	5.12 <sup>bc</sup>
16x22	4.04 <sup>hij</sup>	4.08 <sup>ghi</sup>	4.36 <sup>efg</sup>	5.41 <sup>a</sup>	5.40 <sup>ab</sup>	5.07 <sup>c</sup>
CV (%)		3.90				
LSD(0.05)		0.28				

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05.

### 3.1.3. Root Dry Weight

Analysis of variance revealed significant (P=0.001) difference in root dry weight of coffee seedlings by the interaction of pot size with biochar to top soil ratio (Appendix Table 1). The maximum root dry weight (2.18g) was recorded for 13x19 cm pot size with one to three biochar to topsoil ratio (Table 3). All pot size interacted with one to three biochar to top soil ratio and 16x22cm, 13x19cm interacted with one to two, one to three, one to four and 2gDAP/pot were significantly higher than other treatments. Similarly, the plot treated with 10x16cm interacted with one to four and 2gDAP/pot was statically similar with the highest responsive treatments. This might be due to amendment by organic media, which improved its physical and chemical condition thus favored to increase in the root dry and fresh weights. Seedlings that were raised in the small pots had limited space for root growth, which consequently affected the general plant growth performance. The differences in root weight with various pot sizes might be due to differences in leaf growth and supply of photosynthetic products to the roots (Adu-Berko *et al.*, 2011). Biochar application encouraged root growth and there by affected seedling growth performance. Pot sizes of 7x13cm, 10x16cm, 13x19cm and 16x22cm with one

to three biochar to topsoil ratio was increased seedling root dry weight of coffee seedlings by 102%, 89.47%, 107.61%, and 106.76% over the same size without amendments, and by 86.66%, 71.42%, 107.61% and 106.66%, over the local control, respectively (Table 3). The nitrogen immobilization hypothesis indicates that, when organic matter with high carbon to nitrogen ratio is incorporated in soil, it might reduce nitrogen availability in the soil. Thus, antagonistically induce root system development by main mechanism related to the behavior of microorganisms in soil. In fact, soil organic matter microbes require both organic carbon and nitrogen in a relatively constant ratio. When C:N ratio of organic matter is above the threshold value, mineral nitrogen could be taken from the soil, incorporated in microbial biomass and temporarily immobilized resulting in to low plant available nitrogen (Bonanomi- *et al.*, 2017). Such hypothesis directly supported the present work, because seedling root growths were inversely related to substrate (C:N ratio) that, in turn, was directly correlated with low nitrogen contents of the leaf tissue analysis (Ajema, *et al.*, 2022) even though nitrogen was high in the soil due to nitrogen immobilization and less availability to the roots.

**Table 3: Root dry weight of coffee seedling as affected by pot size and biochar to top soil ratio**

Biochar ratio						
Pot size (cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	0.97 <sup>f</sup>	1.21 <sup>ef</sup>	1.21 <sup>ef</sup>	1.96 <sup>ab</sup>	1.52 <sup>de</sup>	1.06 <sup>f</sup>
10x16	0.95 <sup>f</sup>	1.56 <sup>cde</sup>	1.65 <sup>bcd</sup>	1.80 <sup>a-d</sup>	1.80 <sup>a-d</sup>	1.88 <sup>a-d</sup>
13x19	1.05 <sup>f</sup>	1.56 <sup>cde</sup>	1.84 <sup>a-d</sup>	2.18 <sup>a</sup>	2.16 <sup>a</sup>	2.08 <sup>a</sup>
16x22	1.05 <sup>f</sup>	1.50 <sup>de</sup>	1.97 <sup>ab</sup>	2.17 <sup>a</sup>	1.91 <sup>abc</sup>	2.06 <sup>a</sup>
CV (%)		6.95				
LSD(0.05)		0.38				

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05.

### 3.1.4. Total Dry Weight

Analysis of variance showed significant (P=0.002) differences due to the interaction effects of the two factors (Appendix Table 2). The largest total dry weight (6.83g) was recorded from pot size of 16x22cm interacted with one to three biochar to

topsoil ratio followed by (6.57g) harvested from pot size of 13x19cm interacted with one to four biochar to top soil ratio. On the other hands, the lowest result recorded from the combined effects of smallest pot size with topsoil only (Table 4). The result was in line with Ibrahim *et al.*, (2015) that report, on total dry

weight of coffee seedling affected by the main effects of pot size. The increased total dry weight of coffee seedlings might be the result of both favorable space provided by pot size and contribution of biochar to enhance the soil acidity and/or fertility of the media. All 7x13cm, 10x16cm, 13x19cm and 16x22cm pot sizes interacted with one to three ratio of biochar to topsoil were increased the total dry weight by 26.41%, 22.88%, 35.80% and 42.00%, respectively over the same size but not amended with biochar

only due to biochar ratio. Similarly, the seedling total dry weight was increased over the local control by 16.42%, 20.28%, 37.21%, and 42.00%, respectively. Correlation analysis also indicated that, the total dry weight of the seedling response was strongly and positively correlated with leaf nitrogen ( $r=0.81^{***}$ ), phosphorus contents ( $r=0.82^{***}$ ), leaf dry ( $r=0.97^{***}$ ) and shoot dry weight ( $r=0.96^{***}$ ) of the seedlings (Ajema *et al.*, 2022).

**Table 4: Total dry weight (g) as affected by biochar ratio and pot size interaction**

Biochar ratio						
Pot size(cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	4.43 <sup>j</sup>	4.80 <sup>ij</sup>	5.07 <sup>fgh</sup>	5.60 <sup>ed</sup>	5.85 <sup>cd</sup>	5.18 <sup>fg</sup>
10x16	4.72 <sup>ij</sup>	4.99 <sup>ghi</sup>	5.20 <sup>fg</sup>	5.80 <sup>cd</sup>	5.87 <sup>cd</sup>	5.38 <sup>ef</sup>
13x19	4.86 <sup>ghi</sup>	4.85 <sup>ghi</sup>	5.36 <sup>ef</sup>	6.60 <sup>ab</sup>	6.56 <sup>ab</sup>	5.97 <sup>c</sup>
16x22	4.81 <sup>hi</sup>	5.08 <sup>fgh</sup>	5.35 <sup>ef</sup>	6.83 <sup>a</sup>	6.57 <sup>ab</sup>	5.92 <sup>cd</sup>
CV (%)			3.62			
LSD(0.05)			0.32			

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at  $P \leq 0.05$ .

### 3.2. Biomass Partitioning of Coffee Seedlings

#### 4.2.1. Leaf Mass Ratios (LMR)

Analysis of variance revealed that, leaf mass ratio of coffee seedling was significantly ( $P=0.003$ ) affected due to the interaction of pot size and biochar to topsoil ratio (Appendix Table 2). The highest (0.650%) leaf mass ratio was recorded for 13x19 cm pot size with one to three biochar to top soil ratio followed by (0.647%) responded for 16x22cm pot size with topsoil amended with 2gDAP/pot. All pot size interacted with one to four biochar to top soil ratio, 13x19cm,16x22cm interacted with 2gDAP/pot was statically higher than the rest treatments. On the other side, the minimum coffee seedling leaf mass ratio (0.59 g/g) was recorded for 7x13cm pot size and one to one biochar to topsoil ratio (Table 5). The

availability of essential nutrients in the correct proportion is a key factor for balanced nutrient uptake and healthy plant growth. The nutrient concentrations of plants vary with nutrient availability, plant species, and growing conditions, time of sampling and acidity status of the soil. In this study, leaf phosphorus contents was higher in biochar amended media as compared to local control and phosphorus concentration was also higher in mineral-fertilized soil, implying that biochar supplied phosphorus to the soil and improved its availability to the seedling roots. The other studies have indicated that, amending *Ferralsols* with biochar and compost lowers leaching, thus, optimizes the availability of phosphorus to plants roots (Agegnehu *et al.*, 2015b).

**Table 5: Leaf mass ratio of coffee seedlings as affected by biochar to top soil ratio and pot size**

Biochar to top soil ratio						
Pot size (cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	0.601 <sup>g-k</sup>	0.592 <sup>k</sup>	0.621 <sup>c-h</sup>	0.601 <sup>g-k</sup>	0.630 <sup>a-f</sup>	0.620 <sup>e-h</sup>
10x16	0.601 <sup>g-k</sup>	0.607 <sup>f-j</sup>	0.621 <sup>d-h</sup>	0.623 <sup>c-h</sup>	0.631 <sup>a-f</sup>	0.610 <sup>e-h</sup>
13x19	0.607 <sup>f-j</sup>	0.598 <sup>h-k</sup>	0.646 <sup>abc</sup>	0.650 <sup>a</sup>	0.635 <sup>a-e</sup>	0.645 <sup>a-d</sup>
16x22	0.622 <sup>c-h</sup>	0.624 <sup>b-f</sup>	0.624 <sup>b-g</sup>	0.627 <sup>a-f</sup>	0.630 <sup>a-f</sup>	0.647 <sup>abc</sup>
CV (%)			2.471			
LSD(0.05)			0.025			

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at  $P \leq 0.05$ .

#### 3.2.2. Shoot Mass Ratio (SMR)

Analysis of variance revealed that, the proportion shoot dry weight to total dry matter yield was highly significantly ( $P=0.010$ ) affected by both factors and their interaction (Appendix Table2). The highest SMR (0.866g/g) was recorded for 16x22 cm

pot size with 2g of DAP/pot. Those plots treated with 16x22cm, 13x19cm, 10x16cm treated with one to three and one to four biochar to top soil ratio, as well as all size interacted with 2g DAP/pot were able to respond statically similar result with the highest mass ratio. On the other hand, the minimum value

(0.780 g/g) was recorded for 7x13 cm pot size filled with topsoil alone (Table 6). The increase in plant height, number of leaves and leaf area might have contributed to improved rate of photosynthesis, which consequently contributed to better growth of the seedling shoot parts and thus, more dry matter partitioned to the shoot part. The lower shoot growth or the higher root growth in the media amended with biochar would be the result of good soil condition for

root penetration and growth, thus, enhanced biomass allocation to enhance the root biomass. High plant growth and yield are function of better shoot and root systems with the latter enhancing more nutrient and water uptake from the soil (Jeremiah *et al.*, 2018). The amount of plant nutrient required by coffee plants might vary depending on several factors like plant growth stage and type of nutrients.

**Table 6: Shoot mass ratio of coffee seedling as affected by biochar to top soil ratio and pot size**

Biochar to top soil ratio						
Pot size (cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	0.780 <sup>ijk</sup>	0.804 <sup>a-d</sup>	0.830 <sup>def</sup>	0.804 <sup>hij</sup>	0.780 <sup>ijk</sup>	0.851 <sup>abc</sup>
10x16	0.815 <sup>fgh</sup>	0.821 <sup>e-h</sup>	0.815 <sup>fgh</sup>	0.861 <sup>ab</sup>	0.861 <sup>ab</sup>	0.861 <sup>ab</sup>
13x19	0.804 <sup>hij</sup>	0.805 <sup>g-j</sup>	0.821 <sup>e-h</sup>	0.855 <sup>a</sup>	0.856 <sup>ab</sup>	0.856 <sup>ab</sup>
16x22	0.840 <sup>bcd</sup>	0.804 <sup>hij</sup>	0.814 <sup>fgh</sup>	0.856 <sup>ab</sup>	0.856 <sup>ab</sup>	0.866 <sup>a</sup>
CV (%)			1.326			
LSD(0.05)			0.018			

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05.

**3.2.3. Root Mass Ratio (RMR)**

The statically analysis revealed that, coffee seedling root mass ratio (g/g) was significantly (P=0.02) affected by the interaction of pot size and biochar to topsoil ratio (Appendix Table 2). Accordingly, the largest RMR (0.216g/g) was recorded for the interaction of 13x19cm pot size with one to three biochar to top soil ratio. All pot sizes treated with one to four biochar to top soil and 16x22cm pot size filled by one to three biochar to top soil ratio were statically similar with the largest one (Table 7). It is well understood that, numerous morphological and physiological mechanisms contribute to the efficient use of nutrients for extensive root system. This allows the exploration of large soil volume, capacity to modify the rhizosphere (enabling it to overcome low nutrients), utilization efficiency of nutrients; the ability to maintain normal

metabolism with low nutrient content in tissues and high photosynthetic rate (Lima *et al.*, 2015). Seedlings raised in the smaller pot had limited space for root growth and this consequently affected root mass ratio and general plant growth. Root mass ratio of the seedling might have also been negatively affected by both high soil acidity occurred in the media without biochar amendments and alkalinity effects of the media amended with high ratio of biochar to top soil like one to one and one to two ratios. The soil, acidity reduces plant growth primarily by impairing root growth that reduces in nutrient and water uptake (Marschner, 2012). Similarly, Marschner (2012) reported a negative and significant correlation between the decrease in shoot to root dry matter ratio in phosphate deficient plant and an increase in partitioning of the carbohydrates towards the root.

**Table 7: Root mass ratio of coffee seedling affected by biochar to topsoil ratio and pot sizes**

Biochar Ratio						
Pot size (cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	0.150 <sup>ijk</sup>	0.152 <sup>h-k</sup>	0.169 <sup>fgh</sup>	0.195 <sup>bcd</sup>	0.208 <sup>abc</sup>	0.148 <sup>ijk</sup>
10x16	0.162 <sup>ghi</sup>	0.178 <sup>d-g</sup>	0.185 <sup>def</sup>	0.190 <sup>cde</sup>	0.211 <sup>ab</sup>	0.142 <sup>jk</sup>
13x19	0.163 <sup>ghi</sup>	0.194 <sup>b-e</sup>	0.179 <sup>d-g</sup>	0.216 <sup>a</sup>	0.214 <sup>a</sup>	0.143 <sup>jk</sup>
16x22	0.159 <sup>hij</sup>	0.195 <sup>b-e</sup>	0.185 <sup>def</sup>	0.214 <sup>a</sup>	0.206 <sup>abc</sup>	0.143 <sup>jk</sup>
CV (%)			6.186			
LSD(0.05)			0.018			

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05.

**3.2.4. Root to Shoot Ratio (RSR)**

The RSR of coffee seedlings was significantly (P = 0.017) affected by the combined effects of the two factors (Appendix Table 2). Pot size 13x19cm interacted with one to four biochar to top soil ratio

exhibited the maximum (0.272g/g) RSR as compared to rest of growing media and all pot size interacted with one to four biochar to top soil ratio have statically higher value similar to the largest value (Table 8). That is due to the enough growth space and

organic amended media that improve aeration, water holding capacity, and pH character of biochar as well as chemical condition of the growing media and thus favor increase in the dry mass of the root. Larger pot size and applying biochar to topsoil (at lower ratio or as a fertilizer) considerably improve the nutrient availability, particularly phosphorus, since it improve soil pH under which maximum availability of the nutrient might be obtained and as a result root growth of coffee seedling was improved. It has been

reported that, root biomass, especially the number of roots and root length, is directly related to the media condition thus contribute to growth and survival in the field after transplanting as well as plant height and basal diameter growth (Mohamed, 2013). Jeremiah *et al.*, (2018) have also indicated that, hybrid coffee seedlings with higher root mass had highly significantly increased plant height and stem inter node length.

**Table 8: Root to shoot ratio (g/g) of coffee seedlings as affected by biochar ratio and pot size interaction**

Biochar to top soil ratio						
Pot size(cm)	Top Soil	1BC:1TS	1BC:2TS	1BC:3TS	1BC:4TS	Ts+2gDAP
7x13	0.177 <sup>ijkl</sup>	0.179 <sup>i-l</sup>	0.204 <sup>f-i</sup>	0.243 <sup>bcd</sup>	0.262 <sup>abc</sup>	0.174 <sup>ijkl</sup>
10x16	0.194 <sup>g-j</sup>	0.217 <sup>d-g</sup>	0.227 <sup>def</sup>	0.236 <sup>cde</sup>	0.268 <sup>ab</sup>	0.161 <sup>l</sup>
13x19	0.195 <sup>g-j</sup>	0.241 <sup>b-e</sup>	0.218 <sup>d-g</sup>	0.227 <sup>def</sup>	0.272 <sup>a</sup>	0.167 <sup>kl</sup>
16x22	0.189 <sup>h-k</sup>	0.243 <sup>b-e</sup>	0.227 <sup>def</sup>	0.268 <sup>ab</sup>	0.260 <sup>abc</sup>	0.167 <sup>kl</sup>
CV (%)			7.53			
LSD(0.05)			0.026			

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05.

**3.2.5. Specific Leaf Area and Leaf Area Ratio**

Specific leaf area of coffee seedling was significantly affected by pot size (P=0.003) and biochar to topsoil application ratio (P= 0.0003), but not due to their interaction (P=0.207). Accordingly, the highest (6.88 cm<sup>2</sup>/g) specific leaf area was registered for 13x19 cm followed by 16x22cm pot size. On the other hand, specific leaf areas were enhanced due to biochar to topsoil application ratio and chemical fertilizer (DAP) application used as the standard check. As a result, highest (7.14 cm<sup>2</sup>/g) specific leaf area was recorded for topsoil amended with 2g DAP per seedling (standard check), followed by one to three (6.98 cm<sup>2</sup>/g) biochar to topsoil ratio

(Table 9). Differences in specific leaf area can be ascribed either to morphological factors (thickness of the leaves, vein structure) or to the chemical composition of leaf biomass (Osama, 2018). Leaf Area Ratio (LAR) of seedling was also significantly affected by pot size (P = 0.0001) and biochar to topsoil ratio (P = 0.0001) but not by their interaction (P=0.164). Accordingly, the highest (53.01cm<sup>2</sup>/g) LAR was responded for 13x19cm pot size. The LAR was enhanced by biochar and chemical fertilizer application to top soil used as the standard check. As a result, the highest (56.34cm<sup>2</sup>/g) LAR was recorded from topsoil amended with 2gDAP/pot, followed by one to three (52.28cm<sup>2</sup>/g) ratio of biochar to topsoil.

**Table 9: Specific leaf area and Leaf area ratio as affected by pot size and biochar ratio**

Pot size(cm)	Specific Leaf Area (cm <sup>2</sup> g <sup>-1</sup> )	Leaf Area Ratio (cm <sup>2</sup> g <sup>-1</sup> )
7x13	6.00 <sup>b</sup>	37.5 <sup>c</sup>
10x16	6.06 <sup>b</sup>	42.91 <sup>b</sup>
13x19	6.88 <sup>a</sup>	53.01 <sup>a</sup>
16x22	6.66 <sup>a</sup>	46.53 <sup>b</sup>
LSD(0.05)	0.57	4.47
Biochar to top soil ratio		
Topsoil only	6.21 <sup>dc</sup>	41.27 <sup>b</sup>
1BC :1 TS	5.52 <sup>d</sup>	41.62 <sup>b</sup>
1BC :2 TS	6.43 <sup>bc</sup>	42.94 <sup>b</sup>
1BC :3 TS	6.98 <sup>ab</sup>	52.28 <sup>a</sup>
1BC :4 TS	6.97 <sup>ab</sup>	48.42 <sup>a</sup>
Ts+2gDAP/pot	7.14 <sup>a</sup>	56.34 <sup>a</sup>
CV (%)	13.48	14.81
LSD(0.05)	0.70	5.47

Mean values followed by the same letter(s) within a row or column are not significantly different from each other at P≤0.05

Appendix Table 1

Source of Variation						
Dependent Variable	Replication	Pot size	Biochar ratio	Pot interacted With Biochar ratio	Error	CV (%)
Leaf dry weight	0.012	0.639***	1.414***	0.104***	0.019	14.14
Stem dry weight	0.004	0.020*	0.203*	0.012 <sup>ns</sup>	0.0073	7.78
Shoot dry weight	0.021	0.848***	2.519***	0.116***	0.030	3.90
Total dry weight	0.041	1.425***	4.740***	0.117**	0.039	3.62
Relative growth rate	0.0003	0.007***	0.029***	0.0005*	0.0002	2.13

Appendix 1. ANOVA for dry biomass, Biomass production and RGR Response to the poly bag size and biochar ratio  
 \*\*\*Very highly significant (P< 0.001), \*\* highly significant (P<0.01), \*Significant (P< 0.05), Ns=non-significant difference.

Appendix Table 2: Pearson Correlation Coefficients among some the interacted plant growth parameter

	N	P	SVI	PH	ShFW	LDW	SDW	ShDW	TDW	RV	RDW
N	1.00										
P	0.82***	1.00									
SVI	0.84***	0.84***	1.00								
PH	0.89***	0.86***	0.92**	1.00							
LDW	0.78***	0.79***	0.83**	0.82***	0.70***	1.00					
SDW	0.72***	0.70***	0.65**	0.73***	0.63***	0.60***	1.00				
ShDW	0.84***	0.84***	0.85**	0.87***	0.74***	0.97***	0.77**	1.00			
TDW	0.81***	0.82***	0.85**	0.85***	0.70***	0.94***	0.73**	0.96**	1.00		
RV	0.53***	0.48***	0.64**	0.50***	0.51***	0.57***	0.41**	0.57**	0.64**	1.00	
RDW	0.52***	0.56***	0.62**	0.59***	0.41***	0.63***	0.43**	0.63**	0.80**	0.61*	1.00

N= Nitrogen, P= Phosphorus, SVI= Seedling Vigor Index, PH= Plant height, LDW= Leaf Dry weight, SDW=Stem Dry Weight, ShDW= Shoot dry wight, TDW = Total Dry weight, RV= Root Volume, RDW= Root dry weight.

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