

Influence of Grass-based Digestate and Biochar on Stem Development and Growth in Peppermint (*Mentha piperita* L.)

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Abstract

The inability of certain economically useful flowering plant species to be propagated from seed and the prevalence of poor and infertile soils has made it necessary to explore other alternative means for rapid plant cultivation and growth. This study documents the effects of activated biochar (AB), and treated liquid and solid anaerobic digestate (TLD, TSD) derived from grass cuttings on stem propagation and growth performance of *Mentha piperita* after 60 days of treatment application. Analysis for nitrogen N, phosphorus P, potassium K and carbon C content of TLD, TSD and AB showed that AB was highest in nitrogen (10.91%), potassium (8.15%) and carbon (78%) content while TSD had the highest phosphorus content (2.1%). Results obtained for growth performance showed significant differences ($P < 0.05$) in all treatment groups. Plants treated with 250g TSD showed highest positive effects on all growth parameters after 30 days of treatment application while plants treated with 250g AB had the highest growth performance after 60 days when compared with other treatment groups. AB had slower but more lasting effects on plant growth due to the time needed for biochar to breakdown into useable forms.

Keywords: Biochar, Anaerobic digestate, *Mentha piperita*, Vegetative propagation, Grass cuttings

1.0 Introduction

The continuous depletion of plant nutrients due to changes in environmental conditions of soils is one of the many reasons for the decrease in growth performance of useful plant species. It becomes even more difficult for plants that can only be cultivated through vegetative propagation (Chauhan *et al.*, 2021). Therefore there is need for the production and application of soil remediating formulations such as biochar and organic based fertilizers on nutrient deficient soils.

Biochar is a rich carbon-based material that is produced by the pyrolytic treatment of biomass (Wang *et al.*, 2019). The procedure for producing biochar often involves subjecting biomass materials to relatively high temperatures of between 350°C and 700°C under controlled or limited oxygen conditions (Cantrell *et al.*, 2012). The beneficial effect of biochar in soil enrichment and amendment practices has attracted its applications in agriculture. Biochar serves as a reliable source of plant nutrient and often constitutes noticeable amount of nitrogen, phosphorous, potassium, calcium and other nutrients depending on the primary material from which it is formulated (Ippolito *et al.*, 2015; Schmid *et al.*, 2017). Biochar formulated from animal wastes are often noted for high nitrogen and calcium content when compared to biochar produced mainly from plant residues (Qambrani *et al.*, 2017). The application of biochar on nutrient depleted soils overtime has led to the reduction of soil density and soil hardness with increased soil aeration, structure and cation-exchange capacity (Laghari *et al.*, 2016; Blanco-Canqui, 2021). Aside from the use of biochar for crop improvement and soil amendment, other soil enriching formulations such as anaerobic digestate have been used due to its promising soil nutrient enriching potentials.

Anaerobic digestate (AD) are concentrated sources of nutrient often derived as residues from biogas production sites obtained after anaerobic digestion of biomass (Fedailaine *et al.*, 2015; Lorick *et al.*, 2020). Anaerobic digestates are enriched with plant nutrients namely nitrogen (N), phosphorus (K), and potassium (P) (Plana and Noche, 2016; O'Connor *et al.*, 2022). The proportions of these nutrients in anaerobic digestate are often dependent on the type of feedstock used. At the end of anaerobic digestion, the digestate is often separated into liquid and solid portions. Findings have shown that treated digestate are suitable sources of organic fertilizers commonly used as a replacement for chemical and inorganic fertilizers (Walsh *et al.*, 2012; Evangelisti *et al.*, 2014). The application of treated anaerobic digestate for crop improvement has led to remarkable increase in the growth of many useful crops (Montemurro *et al.*, 2010; Möller and Müller, 2012). The effectiveness and relevance of using anaerobic digestate as suitable replacements for chemical fertilizers has prompted more research in this area. This is due to the side effects of inorganic fertilizers on beneficial soil microbiome with potential negative impact on climate change and habitat disruption of soil micro fauna (Cai *et al.*, 2017; Fang *et al.*, 2021).

Despite the utilization of AD as organic fertilizers and biochar formulations for soil enrichment and growth of many economic crops, little has been documented on using these two nutrient-rich organic materials as growth stimulators for vegetative propagated plant species such as peppermint known for its rich phyto-nutrients in nutraceutical industries.

As part of finding climate friendly alternatives for plant growth enhancers, many organic based formulations have been produced and utilized in recent times for the cultivation of plants with high economic value, most especially plant varieties that are from a hybrid cross which are often sterile or seedless. *Mentha piperita* belongs to this group of highly beneficial plant varieties that are difficult to propagate through seeds. To achieve climate smart agricultural practices; it is important to take advantage of the abundant supply of biomass waste such as grass cuttings for the production of nutrient rich formulations that can enhance plant growth and productivity.

Mentha piperita is a highly valued medicinal plant from the mint family and a product of a hybrid cross between the spearmint and watermint (Shah and Mello, 2004; Balakrishnan, 2015). It is classified under the *lamiaceae* family and it is difficult to cultivate from seeds. This is due to the sterility of the hybrid plant (Vasile *et al.*, 2011; Fayed, 2019). Considering the high commercial value of peppermint plant as feedstock for menthol oil extraction and

other valuable phytochemicals, effective cultivation practices are needed to meet growing demands (Dai *et al.*, 2010; Li *et al.*, 2022). However, this can only be achieved through vegetative propagation techniques.

Vegetative propagation of sterile hybrids and plants species with low seed germination potentials have proven to be a better cultivation technique for large commercial quantities especially with the right growth promoting substrates or soil nutrient additives. This has assisted in meeting the demands for their products and reducing the consequential shortage in supply due to low seeds production (Bisognin, 2011; Awotedu *et al.*, 2021). Also vegetative propagation helps to maintain the breeding of hybrid plants with desired qualities from the parent plant (Riov *et al.*, 2020; Bowman *et al.*, 2021). The aim of this study is to determine the effect of using biochar and anaerobic digestate from grass cuttings as growth promoters in vegetative cultivation of *Mentha piperita*.

2.0 Materials and Methods

2.1 Preparation of Biomass Feedstock for Anaerobic Digestion

Grass cuttings from mown grassland were collected and carefully separated from sand debris and other unwanted materials. The grass cuttings were further reduced into smaller fractions using a grinding machine. This was done to enhance microbial activity by anaerobic microorganisms (Bascaro *et al.*, 2015). Bio-digester (80 litres) was constructed using a plastic drum with an outlet pipe to allow the expulsion of biogas into an airbag. 20kg ground grass clipping was measured using a weighing balance and was transferred into the 80 litres capacity bio-digester. Hot water (100°C) was added to the feedstock at ratio 1:2 and was used for the final preparation of feedstock for anaerobic digestion. The initial pH of 6.8 was obtained using a pH/ion meter (GLP 22) after which the feedstock was allowed for anaerobic digestion to take place for a period of 60 days with close monitoring of the digestion process.

2.2 Collection and Treatment of Anaerobic Digestate

Collection of anaerobic digestate was carried out after 60 days. The collected digestate was transferred into a steel drum and thermally treated for 2hrs at 100°C. This was done to reduce pathogens and stabilize the digestate for use as plant growth promoter. The thermally treated digestate was allowed to cool for 1hr and was filtered through a 20 micron interwoven wire mesh. The solid portion of the treated digestate was air dried for 48hrs at atmospheric temperature under controlled environment while the liquid digestate was stored in an air tight plastic container to prevent possible contamination by external factors before use.

2.3 Production of Biochar from Anaerobic Digestate

Part of the dried solid treated digestate was used for the production of biochar through pyrolysis under 300°C using a pyrolytic chamber (PC). The solid digestate (SD) was transferred into an inner loading chamber of the pyrolytic chamber and was sealed to avoid the entrance of oxygen. The outer chamber had an outlet made of steel pipe that allows gases produced during the process of pyrolysis to be dispersed. Pyrolysis of the solid grass digestate was only done for one hour since the lignin content in grass is low and had already been broken down during the process of anaerobic digestion. The carbonised SD was collected from the PC after cooling. The carbonised SD was measured (10kg as total weight)

and activated by steeping in 5 litres of cow urine after which it was allowed to stand for 20 days to activate microbial activity.

2.4 Preparation of Soil Samples and Different Concentrations of Growth Promoters (Biochar and Treated Digestate)

A total of 56 medium sized polythene planting bags were filled each with 2kg of soil. The soil samples were watered and kept in a screen house. Thereafter, the soil pH was noted prior to the application of growth promoters. Different concentrations of growth promoters were prepared from activated biochar and treated digestate (solid and liquid). 125ml/L and 250ml/L of treated liquid digestate (TLD) was prepared by measuring 125ml and 250ml TLD each into containers containing 1 litre of tap water while 125g and 250g of treated solid digestate (TSD) and activated biochar (AB) was measured respectively using a digital weighing balance. The controlled group constituted only tap water.

2.5 Analysis of Biochar and Anaerobic Digestate for Plant Nutrient Content before Treatment Application

Analysis of the soil sample and the treatment materials (TLD, TSD, AB) were carried out to determine the Nitrogen (N), phosphorous (P), potassium (K) and carbon (C) contents using the Kjeldahl method for total nitrogen analysis (Abrams *et al.*, 2014), coupled plasma atomic emission for total phosphorus and potassium content (Yang *et al.*, 2002) and analysis for total organic carbon (Jarvie, 1991).

2.6 Collection and Preparation of Plant Stem Cuttings

Plant samples of *Mentha piperita* were gotten from a known breeder in Calabar Municipality, Cross River State, Nigeria after which the plant samples were taken to the herbarium laboratory at the Department of Plant and Ecological Study, University of Calabar, Nigeria for proper identification. A total of 56 stem cuttings of *Mentha piperita* were obtained from the parent plant using a sharp thin-bladed pocket knife. The stem cuttings were taken to the greenhouse for vegetative propagation.

2.7 Experimental Design and Treatment Application

The randomised complete block experimental design (RCBD) was used in this study with each treatment group having 8 replicates, making a total of 56 treatment units of vegetative propagated *Mentha piperita* (stem cuttings). All treatment groups had varying concentrations of treated digestate according to the treatment schedules. Plant samples in all treatment groups were watered with tap water every four days. Plant samples in the control group had no other treatment application aside from watering the plants every four days. Treatment of plant samples with TLD, TSD and AB was done before vegetative propagation of *Mentha piperita* with second treatment application after 20 days of planting (DAP).

2.8 Collection of Data

Data collection was carried out using the following growth parameters; number of leaves, plant height, number of branches and leaf area. The number of leaves and branches per plant was determined by counting the leaves and branches produced from each plant. Plant height and leaf area was measured with a meter rule. Data on these parameters were taken twice

during the growing season at 30 and 60 DAP. Data obtained were subjected to statistical analysis of variance at a 5% probability level.

3.0 Results

3.1 Nitrogen, Phosphorus, Potassium and Organic Carbon contents of the Treated Digestate and Activated Biochar

Results obtained from the analysis for concentration of nitrogen N, phosphorus P, potassium K and organic carbon C content in soil samples, treated with TLD, TSD and AB are shown in table 3.1 below.

Table 3.1: Percentage N P K and C concentration in treated liquid digestate, treated solid digestate, activated biochar and soil sample used to cultivate stem cuttings of *Mentha piperita* L.

Treatment	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Carbon (C)
TLD	5.6	0.9	3.6	37
TSD	7.9	2.1	5.8	48
AB	10.91	0.2	8.15	78
Soil sample	1.4	0.01	1.1	15

NB: TLD-Treated liquid digestate, TSD-Treated solid digestate, AB-Activated biochar

3.2 Effect of Treated Digestate and Activated Biochar on Plant Height

The plant height after treatment application of both liquid and solid treated digestate and activated biochar showed significant increase in all treatment groups. Results obtained showed that plants treated with 250g of TSD had the highest mean plant height of 14.6cm followed by plants treated with 250g AB with a mean plant height of 12.8cm. Plants treated with 125ml TLD showed the least significant increase in plant height when compared to the control group as shown in figure 3.1. Further evaluation of the effects of different treatment application of TLD, TSD and AB after 60 days showed that plants treated with 250g AB outgrown all other plants in various treatment groups with a mean plant height of 22.1cm. As shown in figure 3.2, it was observed that plants treated with 250ml/L TLD and 125g TSD had the same mean plant height of 10.4 while plants treated with 250g TSD and 125g AB had the same mean plant height of 13.7. However, all treatment groups showed significant increase in plant height when compared to the control group.

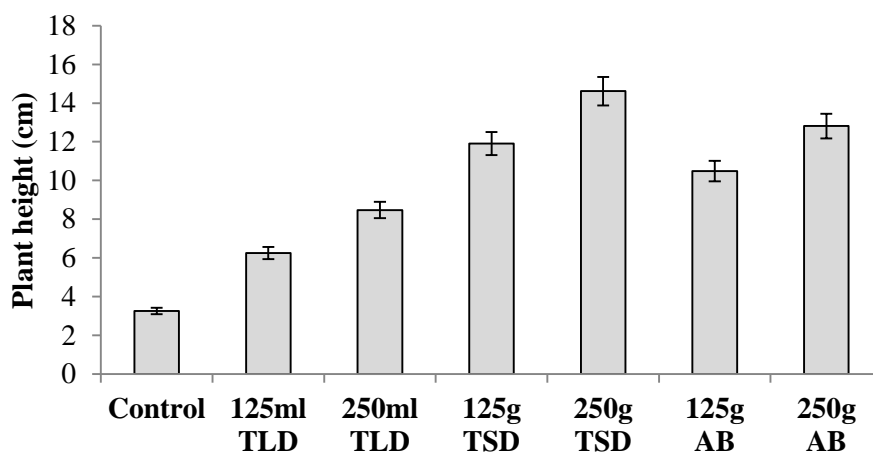


Figure 3.1: Mean plant height of *Mentha piperita* L after 30 days of propagation on soil treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

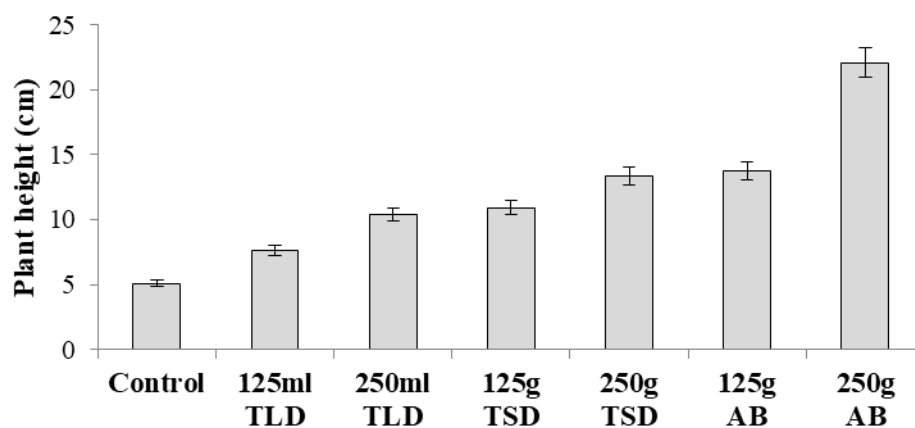


Figure 3.2: Mean plant height of *Mentha piperita* L after 60 days of propagation on soils treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

3.3 Effect of Treated Digestate and Activated Biochar on the Number of Leaves

Number of leaves produced by plants in all treatment groups after 30 days of treatment application showed significant increase in all treatment groups when compared with the control group. Groups treated with 125ml/L of TLD, 250ml/L of TLD, 125g of TSD, 250g of TSD, 125g of AB and 250g of AB had the following mean number of leaves; 15.25, 25.75, 32.5, 46.8, 25.5 and 32.7 respectively. However, plants treated with 250g TSD produced the highest number of leaves at 30 DAP (figure 3.3). Also data obtained after 60 DAP (Figure 3.4) maintained a significant increase in the number of leaves when compared to the control group. Plants treated with 250g AB and 250g TLD had the highest number of leaves of 90

and 86 when compared with the control group as shown in figure 3.4, while plants treated with 125ml TLD had the lowest increase in the number of leaves of 27.5 amongst the treated groups.

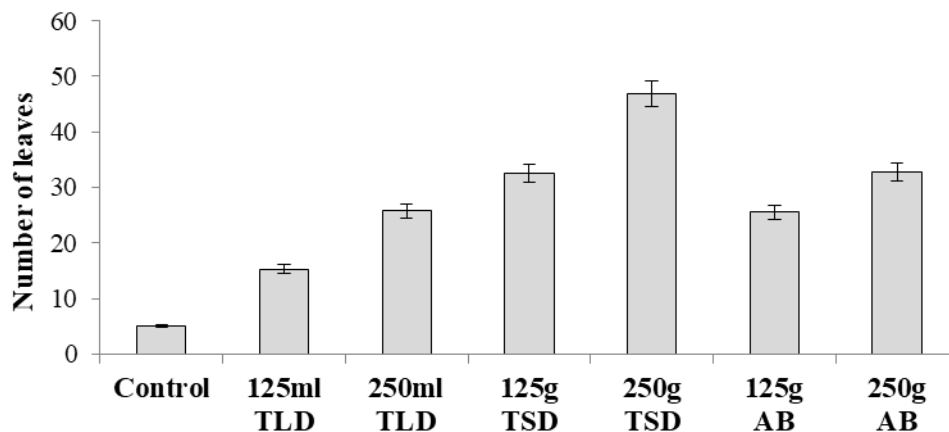


Figure 3.3: Mean number of leaves of *Mentha piperita* L after 30 days of propagation on soil treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

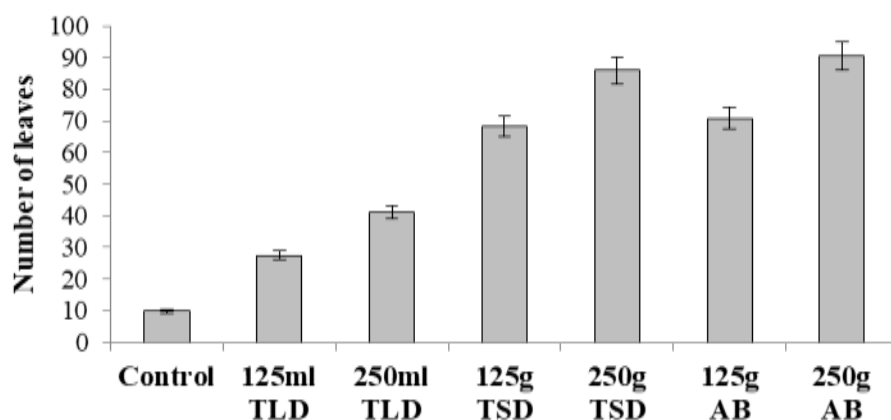


Figure 3.4: Mean number of leaves of *Mentha piperita* L after 60 days of propagation on soil treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

3.4. Effect of Treated Digestate and Activated Biochar on the Number of Branches: The number of branches for each plant in all treatment groups showed significant effect of TLD, TSD and AB on the growth performance of *Mentha piperita*. As shown in figure 3.5, plants treated with 250g TSD had the highest mean of 8.8 branches followed by plants treated with 250g AB with a mean of 7.2 branches. Every other treatment group had a mean above 1 except for the control group. After 60 days of treatment application, plants treated with 250g of TSD maintained the highest number of branches but with an increased mean number of 12

branches (figure 3.6). Plants treated with 250g AB had about 10 branches while plants treated with 125g TSD and 125g AB had the same number of branches (7) which is higher than what was obtained after 30 days of treatment application as shown in figure 3.6. Also plants treated with 125ml of TLD had 1 additional branch while plants treated with 250ml TLD had 2 additional branches at 60 DAP.

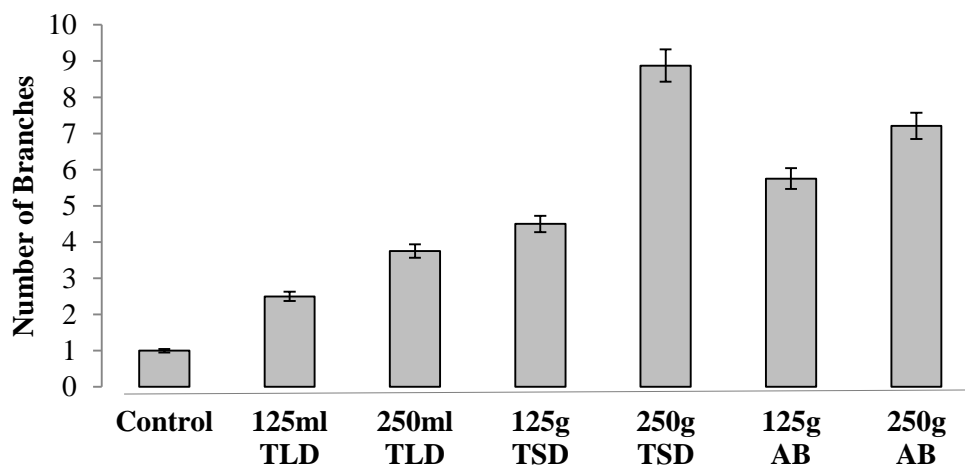


Figure 3.5: Mean no of branches of *Mentha piperita* L after 30 days of propagation on soils treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

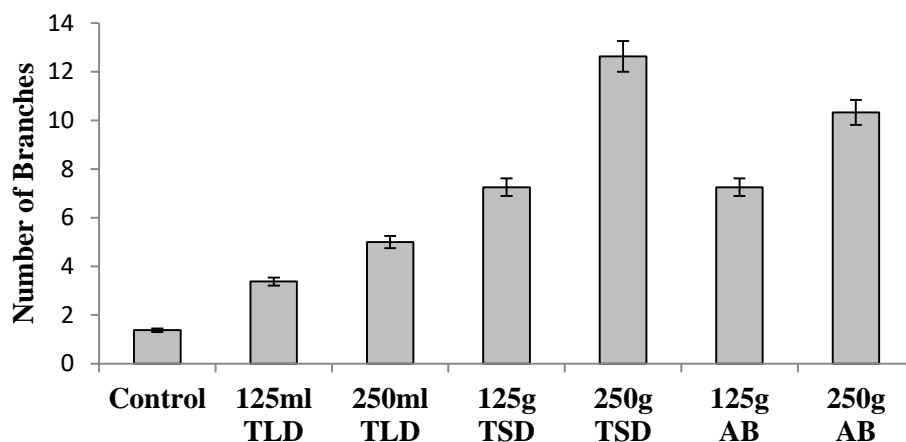


Figure 3.6: Mean no of branches of *Mentha piperita* L after 60 days of propagation on soils treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

3.5. Effect of Treated Digestate and Activated Biochar on Leaf Area

The effect of TLD, TSD and AB on the mean leaf area of *Mentha piperita* indicated that plants treated with 125ml TLD, 250ml TLD, 125g TSD and 125g AB had the same mean leaf area of 0.8 m² while plants in the control group had a mean leaf area of 0.7 m². However, 250g TSD and 250g AB produced the leaf areas of 2.1 m² and 1.5 m² respectively (figure 3.7). Results obtained after 60 days of treatment application showed an increase in leaf area in the 250g AB treatment (2.2 m²) when compared with other treatment groups. Plants treated with 250ml TLD and 125g TSD had the same leaf area but with a slightly higher value of 0.9 m² when compared with what was obtained after 30 days. Plants treated with 125ml TLD and 250g TSD were 0.8 m² and 2.1 m² respectively (figure 3.8).

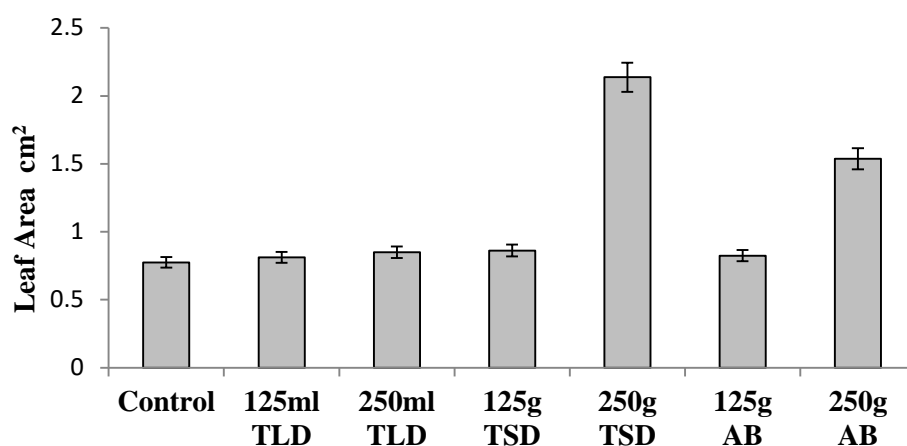


Figure 3.7: Mean leaf area of *Mentha piperita* L after 30 days of propagation on soils treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

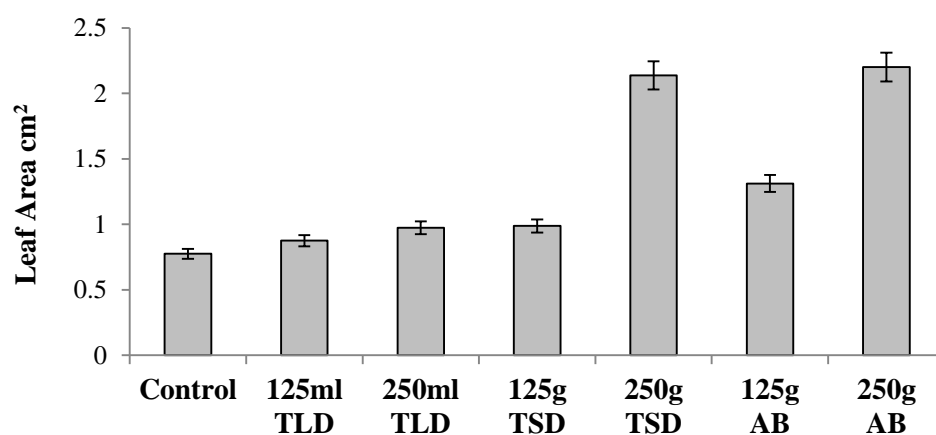


Figure 3.8: Mean leaf area of *Mentha piperita* L after 60 days of propagation on soils treated with activated biochar (AB), liquid and solid anaerobic digestate (TLD, TSD)

4.0 Discussion

Analysis for nitrogen (N), phosphorus (P), potassium (K) and carbon content showed activated biochar (AB) had the highest nitrogen, potassium and carbon content. This is probably due to its activation with cow urine which is a good source of nitrogen. Urine from livestock incorporated into organic manure are a potential resource for crop improvement and productivity (Devasena and Sangeetha, 2022; Ekwere *et al.*, 2023). Sutradhar *et al.*, (2021) reported that animal urine-enriched biochar had a 60% yield increase in cultivated cabbage and kohlrabi crops. Also, results obtained for growth performance of treated plants showed significant increase in all growth parameters for each treatment groups when compared with the control group. However, the application of treated liquid digestate, treated solid digestate and activated biochar showed an array of varying growth promoting effects for each growth parameter under study. This is to suggest that these formulations affected the plant growth differently.

Considering the outcome of all treatment applications on the four growth parameters, it was observed that plants treated with 250g of TSD had a good vegetative start and showed the highest plant growth performance after 15 days of treatment application for all growth parameters. This shows that vegetative cultivated plants are likely to thrive well under good concentrations of TSD and also depicts that 250g TSD enhanced the vegetative propagation process than any other treatment applications thus giving the plants better chances of surviving vegetative propagation from plant's stem cuttings. This might be due to its high phosphorus content when compared with other treatments. According to Jurgutis *et al.*, (2021) lignocellulosic solid digestate showed significant vegetative growth on treated *Brassica juncea* plants. This is in confirmation with the findings obtained in this study. It was also observed that plants treated with TSD performed better than plants treated with TLD in all growth parameters. This is because the nitrogen and phosphorus composition of TSD is often greater than TLD and also, liquid based digestate are known to contain lower concentrations of these two plants nutrients when compared to solid digestate. A similar report was documented by Olugbemide and Likozar, (2022). The outcome of their study showed higher concentrations of nitrogen and phosphorus in rice husk solid digestate than the liquid digestate. Interestingly, nitrogen is essential for greenish and healthy leaf production while phosphorus enables root formation in plants (Chrysargyris and Panayiotou, 2016). This is likely the reason why plants treated with 250g TSD performed better during the first 30 days of cultivation.

Further evaluation of results obtained in this study showed that plants treated with biochar did not thrive well for the first 30 days of vegetative propagation. According to Joseph *et al.*, (2021) there are three reaction stages of biochar namely; the dissolution stage which takes 1 – 3 weeks, the reactive surface development which is between 1 – 6 weeks and lastly the aging stage which is above 6 months. This report suggests that it is unlikely to see the beneficial effects of biochar within the first 1 – 4 weeks hence the outcome of this study during the first 30 days. The dissolution stage involves the breaking down of biochar into useable form thus releasing nutrients for plant growth (Yang *et al.*, 2021). The dissolved biochar often constitute dissolved salts, minerals and organic matter (Sun *et al.*, 2021). However, plants treated with 250g biochar were noted for achieving the highest growth performance in all growth parameters after 60 days except for number of branches in which plants treated with 250g TSD were highest. However, plants treated with 250g biochar were noted for achieving the highest growth performance in all growth parameters after 60 days. This suggests that the beneficial effect of biochar is slow but most effective overtime when compared to treated solid and liquid digestate. Plants treated with a lower concentration of biochar (125g) showed

minimal growth enhancing potentials which suggest that a 250g biochar application is more effective and reliable for better plant growth improvement.

4.0 Conclusion

In this study, the use of anaerobically treated grass biomass waste in the form of liquid/solid digestate and activated biochar was evaluated for its potentials as an alternative source for plant nutrients through its application on vegetative propagated *Mentha piperita* over a period of 60 days. This research has been able to evaluate the potential benefit of using treated anaerobic digestate and activated biochar for the vegetative propagation of *Mentha piperita* and the results obtained showed these formulations can be used for vegetative propagation. Findings from this study suggest treatments with 250g TSD and 250g AB as the most recommended treatments due to high growth performance with these treatment applications. However, more study is required to evaluate its beneficial effect on other vegetative cultivated plant species to further ascertain its usefulness in sustainable plant cultivation and production.

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