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Effect of biochar on growth and yield of cabbage (*Brassica oleracea* var. *capitata* L.) in coastal region

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ABSTRACT

The process of heating biomass (wood, manure, crop residues, solid refuse, etc.) with little to no oxygen in a furnace that has been specifically designed to capture all emissions, gases, and oils for later use as energy results in biochar, which is fine-grained charcoal. The capacity of biochar to draw in and hold water, nutrients, phosphorus, and agrochemicals is superior to that of any other organic soil material. Less fertilizer leaches into groundwater and flows off into surface waters, and plants are healthier as a result. The head of cabbage (Brassica oleracea var. capitata) is a small, leafy biennial that produces a tightly packed globular mass of smooth or crinkled leaves coiled around one another. The cultivation of cabbage benefits greatly from the use of biochar. Using biochar reduces the cost to the farmer because it eliminates the need for water irrigation and chemical fertilizer. The experiment was conducted to evaluate the effect of biochar on growth and yield of cabbage (Brassica oleracia var. capitata) at the Research Field of the Department of Agriculture, Noakhali Science and Technology University (NSTU), Sonapur, Noakhali-3814. The biochar was added to the soil at 0, 3.0, 6.0, 9.0 and 12.0 tons per hectare, respectively. The treatments were arranged in a Randomized Complete Block Design with 3 replications from mid-January 2022 to mid-April 2022. Significant variations ($p < 0.01$) for all the parameters were revealed in the study. According to the study, the highest plant height was (19.92 cm), leaf length (26.12 cm), leaf breadth (23.14 cm), number of unfolded leaf (19.12 cm), weight of largest leaf (34.00 g), head diameter (15.14 cm), head width (13.00 cm), weight of head (765 g), dry weight of head (91.30 g), stump length (10.24 cm), stump diameter (2.20 cm), root length (21.00 cm) and chlorophyll content (73.00). The result indicates the good performance of wood biochar in cabbage production. The maximum growth and yield of cabbage come from T_5 (12.0 t ha^{-1}). The experiment suggested that T_5 (12.0 t ha^{-1}) is the best for improvement in mostly edible cabbage crops during winter season in Bangladesh.

Key Words: *Biochar, Cabbage, Weight of head, Yield and Chlorophyll content.*

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I. Introduction

Cabbage (*Brassica oleraceae* var. *capitata* L., 2n=18) is globally recognized as one of the most important leafy vegetables within the Brassicaceae family (Adeniji et al., 2010). Originating in Western Europe, cabbage holds a significant place in human diets due to its widespread availability, affordability, and nutritional richness (Silva Jr et al., 1986). Its high content of ascorbic acid, calcium, and potassium contributes to its nutritional value (Singh et al., 2006; Higdon et al., 2007; Leja et al., 2007; Šamec et al., 2011). In Bangladesh, cabbage is an essential and nutritious winter vegetable. Winter vegetable consumption has increased in recent years and it significantly boosts the country's economy (Ali et al., 2023). Despite its importance, cabbage yield per unit area in Bangladesh remains relatively low compared to developed nations (Schippmann et al., 2002).

On the other hand, biochar a fine-grained charcoal rich in organic carbon-has emerged as a promising soil amendment due to its resistance to decomposition and beneficial effects on soil properties (Joseph et al., 2012). Produced through the pyrolysis of plant and waste feedstocks (Liu et al., 2016; Zhang et al., 2019; Wang and Wang, 2019), biochar is characterized by a low O/C molar ratio and stability against biological and chemical degradation (Spokas et al., 2010; Song et al., 2012). Its high cation-exchange capacity and alkaline nature make biochar suitable for enhancing soil fertility (Lehmann et al., 2011). However, the chemical composition and stability of biochar can vary significantly depending on the source materials and production conditions (Kookana et al., 2011; Nartey et al., 2014).

In agricultural contexts, biochar amendment has shown the potential to improve soil biological properties by providing biodegradable organic carbon and creating favourable habitats for soil microbial activities (Kuzyakov et al., 2014; Rahaman et al., 2023). Moreover, biochar offers a low-cost solution for addressing soil fertility depletion and has been linked to increased agricultural productivity (Atkinson et al., 2010). Its ability to adsorb large quantities of salt further enhances its value as a soil additive (Puja, 2021).

Despite the growing interest in biochar globally, its adoption and application in Bangladesh remain relatively novel (Sutradhar et al., 2021). Limited research has been conducted on biochar's effects on agricultural residues at field scales, underscoring the need for comprehensive investigations to highlight its significance and potential in Bangladeshi soils (Weyers et al., 2014; Abdulaha-Al Baquy et al., 2022). Therefore, a thorough investigation of biochar research is required to highlight the significance, scope, and opportunities of its implementation in Bangladeshi soil. This study explores the application of biochar in large-scale cabbage cultivation, aiming to evaluate its impact on growth and yield parameters under local conditions.

II. Materials and Methods

Experimental location and climate

The research was carried out at the Research Field of the Department of Agriculture, Noakhali Science and Technology University (NSTU), Sonapur, Noakhali-3814, from December 2021 to March 2022. The Agro-Ecological Zone of the research area was the Young Meghna Estuarine Floodplain (AEZ-18) (FAO, 1988), which was under coastal region. The research field was situated in the tropical climatic zone where average high temperature was 25.8-33.8°C from January 2022 to April 2022.

Soil of experimental site

The soil of experimental site was sandy loam in texture, medium high land. The experimental field given soil $P^{H,}$ moisture, Electrical conductivity, Total Organic Carbon, Total Organic Matter presented in Table 01.

Experimental design

The single factor experiment was set up using a Randomized Complete Block Design with three replications (RCBD). The experiment was conducted during Rabi season. In this experiment, no. of treatments were 5 and number of replications were 3. Seedling spacing between row to row and plant to plant was 60 cm*45 cm and plot size was 0.54 m^2, plot spacing 30 cm and total plot number was 15. Total experimental area was 14.04.

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Five different doses of biochar T_1 = no biochar, T_2 = biochar (3 t ha^{-1}), T_3 = biochar (6 t ha^{-1}), T_4= biochar (9 t ha^{-1}), T_5 = biochar (12 t ha^{-1}) were used in the experiment as treatment.

Table 01. Physio-chemical properties of the soil before experimenting.

SL No.	Parameters	Unit	Results	Reference test method	Remarks
1	PH	-	7.42	PH Meter	-
2	Moisture	%	1.56	IS I 2720(part II) 1973	-
3	Electrical conductivity	µS/cm	111	Conductivity Meter	-
4	Total Organic Carbon	%	1.37	Walkley-Black titration Method	-
5	Total Organic Matter	%	2.36	Walkley-Black titration Method	-

Land preparation

The experimental plots were ploughed, harrowed, and leveled. Three times of harrowing were used to pulverize the soil, followed by laddering to ensure good tilth. All weeds and trash from the previous crop were removed, and the field was finally prepped with a base dose of cow dung. According to Agriculture Information Service (AIS), recommended fertilizer dose of Urea 247 kg ha^{-1}, TSP 197 kg ha^{-1}, MoP 160 kg ha^{-1}. The total amount of TSP in the form of triple superphosphate was applied at the time of final land preparation. Urea and MoP fertilizers were applied in 2 installments, once after 20-25 days of transplanting and once more after 30-40 days.

Collection of seedlings

The crop was cabbage and variety was Atlas 70. Seedlings (30 days) of cabbage variety (atlas 70) were collected from online shop 'Seed Bazar' and Healthy seeds were selected for cultivation.

Biochar application

Biochar was collected from Christian Commission for Development in Bangladesh (CCDB) Mirpur 10, Dhaka, Bangladesh and a full amount of biochar was applied during final land preparation before transplanting of seedlings. Then, very little amount of water was sprinkled over the plot once.

Transplanting of seedlings

The 32-day seedlings among the varieties were transplanted on 13th January and maintained spacing 45 cm × 60 cm (plant to plant and row to row) at the rate of six seedlings per plot.

Intercultural operations

The following intercultural operations were done to ensure and maintain the normal growth of the crops. Regular weeding was carried out on the field to prevent crop weed competition, infestation of pest and diseases and to ensure maximum growth of crops. First weeding was carried out after 10 days of land preparation and another was 5 days interval. Watering was done at 5 to 7 days interval after transplanting. Irrigation pipes were used for these purposes. The method of pesticide application depends on nature of pests, site of application, type of pesticide formulation etc. Docord10EC (Cypermethrin) @ 1.25 ml per liter was sprayed to control insects. The rate of application of pesticide should be uniform over the whole of the field area. Continuous observation was done to ensure better growth of plants for good yield. Insect attack was not severe. Disease's infestation was not too severe to cause damage to the crop. Harvesting of cabbage was done at marketable stage by uprooting the whole plant along with roots. Harvesting was done in morning hour.

Data collection

The data were collected after harvesting to obtain various physio-morphological and biochemical traits. Data collection was done on the following parameters i.e., Plant height, Leaf length (largest leaf), Leaf breadth (largest leaf), Number of unfolded leaves, Weight of largest lea, Head diameter, Head width, Weight of head, Dry weight of head, Stump diameter, Stump length, Root length and Chlorophyll content.

Statistical analysis

For data analysis of the collected data, IBM SPPS statistics, version 26, was used to perform an analysis of variance (ANOVA) and the figures were drawn using Microsoft Excel 2016. According to the guidelines provided by Gomez and Gomez (1984), the mean differences between the treatments were determined using Tukey HSD of post-hoc analysis were performed.

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III. Results and Discussion

Plant height

The analysis of variance showed that the application of biochar doses had a significant effect on the height of cabbage plant. It clearly shows that T_5 significantly increased the plant height at 15 DAT, 35 DAT and 60 DAT compared to untreated control. The tallest plant was found to be 8.44 cm from T_5 at 15 DAT, 18.10 cm at 35 DAT, 19.92 cm at 60 DAT. On the contrary, the lowest values of 7.00 cm at 15 DAT, 13.00 cm at 35 DAT, 17.10 cm at 60 DAT were recorded from T_1 (Figure 01). The increase in cabbage plant height observed in this study underscores the potential of biochar as a sustainable soil amendment for enhancing crop productivity. In support of our result, several studies stated that biochar treatment in a cabbage increased plant height (Carter et al., 2013; Balkaya et al., 2005).

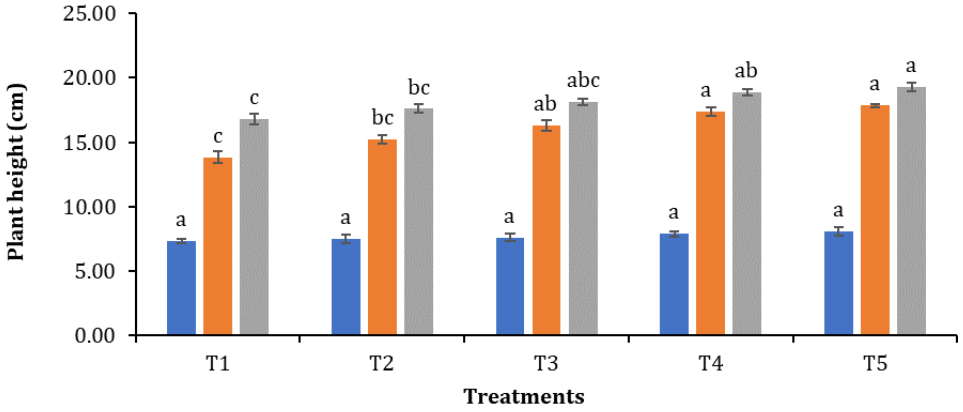

Figure 01. Effect of different biochar doses on plant height at 15, 35, and 60 DAT.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant differences (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Leaf length

Largest leaf length of cabbage varied significantly among treatments on different days after transplanting (DAT). Based on recorded largest leaf length at 15, 35 and 60 DAT, T_5 showed significantly increased leaf length. The highest leaf length was found to be 5.00 cm from T_5 at 15 DAT, 20.10 cm at 35 DAT and 26.12 cm at 60 DAT. On the contrary, the lowest values of 4.03 cm at 15, 16.67 cm at 35 and 22.50 cm at 60 DAT were recorded from T_1 (Figure 02). According to McDonald et al. (2019) biochar, with its unique properties such as high porosity and nutrient retention capacity, facilitated enhanced nutrient availability and water retention in the soil, thereby promoting optimal growth conditions for cabbage plants. Also, A massive number of studies were indicated to determine the effect of biochar application on leaf length of cabbage plants (Sun et al., 2021).

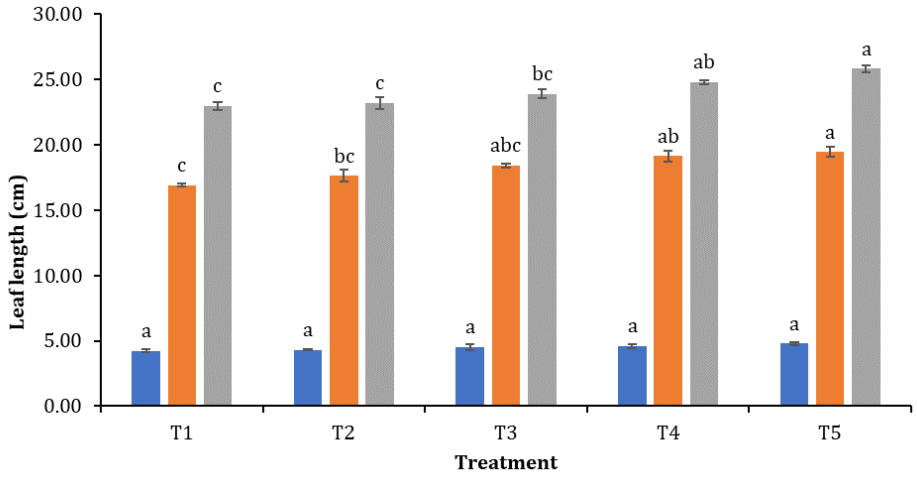

Figure 02. Effect of different biochar doses on leaf length (largest leaf) at 15, 35, and 60 DAT.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

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Leaf breadth

Largest leaf breadth of cabbage varied significantly among treatments at different days after transplanting (DAT). Based on recorded largest leaf breadth at 15, 35 and 60 DAT, T_5 showed significantly increased leaf length. The highest leaf breadth was found to be 4.17 cm from T_5 at 15 DAT, 14.10 cm at 35 DAT and 23.14 cm at 60 DAT. On the contrary, the lowest values of 3.30 cm at 15, 10.63 cm at 35 and 18.83 cm at 60 DAT were recorded from T_1 (Figure 03). The result revealed that biochar improved leaf breadth of cabbage plants which was also in line with the results observed by another researcher (Harris et al., 1998).

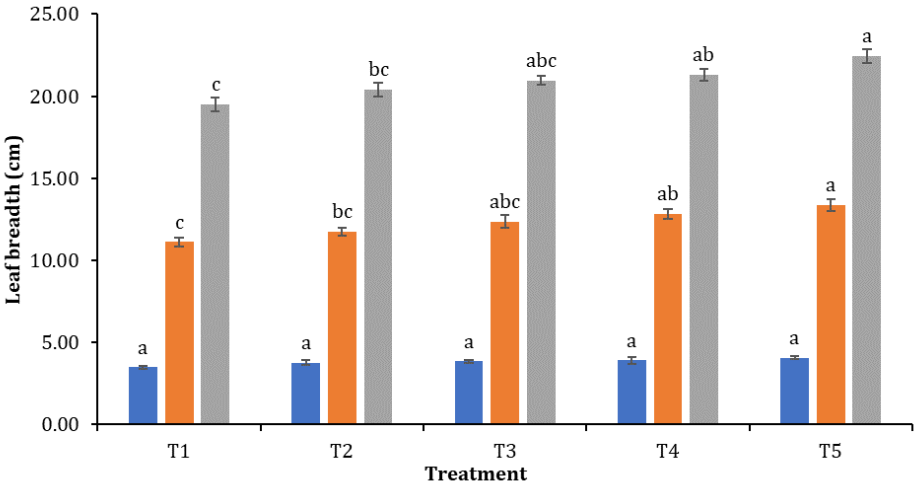

Figure 03. Effect of different biochar doses on leaf breadth (largest leaf) at 15, 35, and 60 DAT.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Number of unfolded leaves

Number of unfolded leaves showed highly significant variation. The experiment's results indicate that number of unfolded leaves was significantly influenced by treatments. The highest number of unfolded leaf (19.12) was found in T_5 (12.0 t ha^{-1}), followed by T_4 (9.0 t ha^{-1}), T_3 (6.0 t ha^{-1}) and T_2 (3.0 t ha^{-1}) whose numbers were successively 17.81, 16.19 and 15.80 and the lowest leaf number (13.69) found in T_1 (control) (Figure 04). Number of unfolded leaves is also an important morphological trait because it is the main photosynthetic part of cabbage. In our study, number of unfolded leaves (13.69-19.12) was recorded which similar result was counted by different studies (Terefe et al., 2018). The development of unfolded leaves influenced by biochar application as biochar's ability to improve soil structure, nutrient retention, and water-holding capacity contributes to enhanced leaf development (Yooyen et al., 2015).

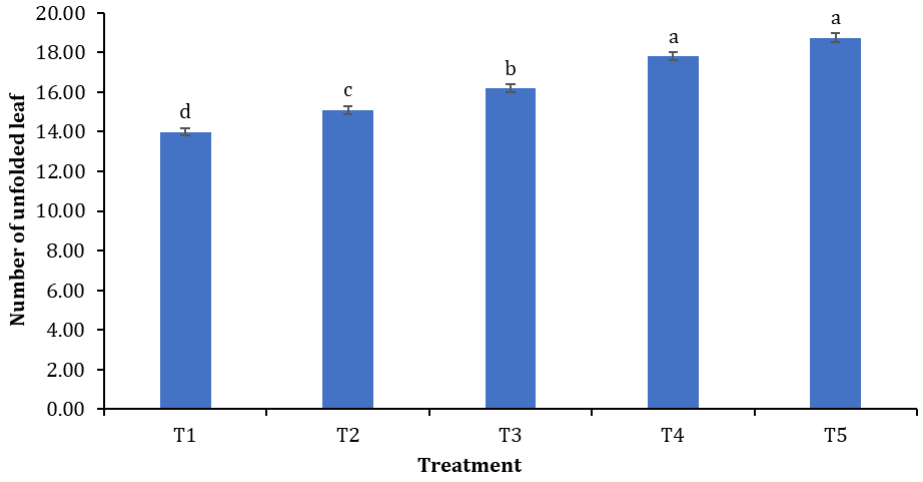

Figure 04. Effect of different biochar doses on number of unfolded leaves.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c, d) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

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Weight of largest leaf

Weight of largest leaf showed highly significant variation. The experiment's results indicate that Weight of largest leaf was significantly influenced by treatment. The highest Weight of largest leaf (34.00 g) was found in T_5 (12.0 t ha^{-1}), followed by T_4, T_3 and T_1 whose weight of largest leaf was successively 31.60 g, 30.23 g and 29.13 g and the lowest leaf width (27.00 g) found in T_1 (control) (Figure 05). According to the study, weight of largest leaf of cabbage statistically increased with the application of biochar which was similar to the result observed in an experiment (Teshome et al., 2020). This suggests a consistent benefit of biochar in promoting leaf development and overall plant vigor. The improved leaf development not only contributes to higher biomass accumulation but also indicates better nutrient uptake and utilization by the plants.

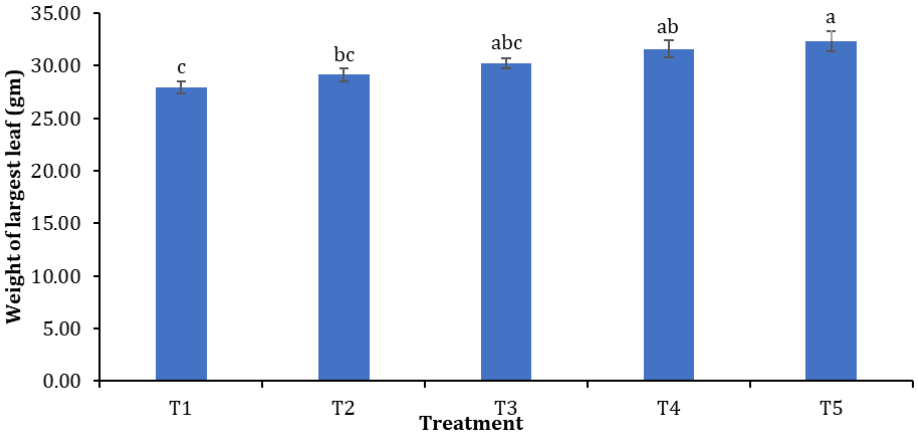

Figure 05. Effect of different biochar doses on weight of largest leaf.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Head diameter

The study showed that biochar has significantly effect on head diameter of cabbage plants. The finding showed highly significant variation. The highest head diameter was found in T_5 (15.14 cm). The lowest head diameter was observed in T_1 (9.36 cm) (Figure 06). This result suggests that biochar application positively influences the head diameter of cabbage plants. Different studies reported that the head diameter of cabbage statistically increased with the application of biochar (Hope et al., 2016). Another study stated that biochar treatment in a cabbage increased head diameter (Teshome et al., 2019).

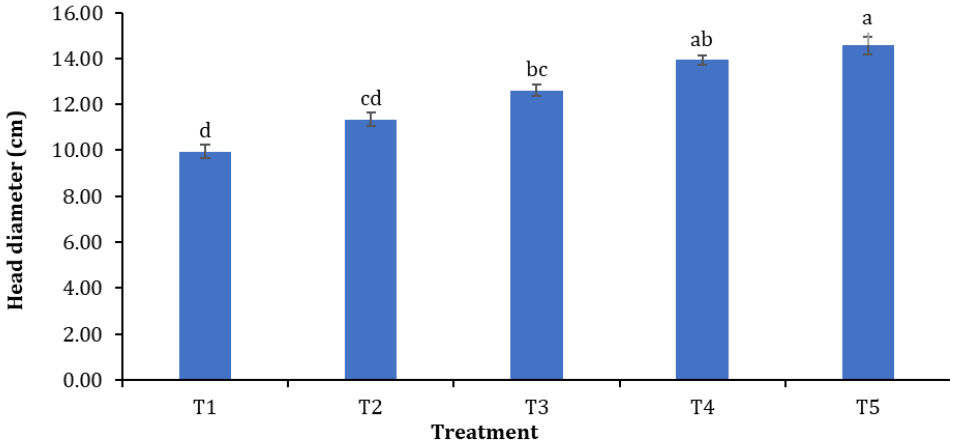

Figure 06. Effect of different biochar doses on head diameter.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c, d) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Head width

In accordance with the study, there was a huge variance ($p < 0.01$) among treatments. The maximum head width was viewed in T_5 (13.00 cm). The lowest head width was found in T_1 (7.81 cm), followed

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by T_2. There was no statistical dissimilarity among the T_3 and T_4 (Figure 07). In case of head width in this study, recorded value was 7.81 cm to 13.00 cm. Similar findings were recorded by different studies (Bhandari et al., 2021). Another study stated that biochar treatment in a cabbage increased head width (Bindu et al., 2019). The significant improvement in head width with higher biochar application rates underscores the potential of biochar as a beneficial soil amendment for cabbage cultivation.

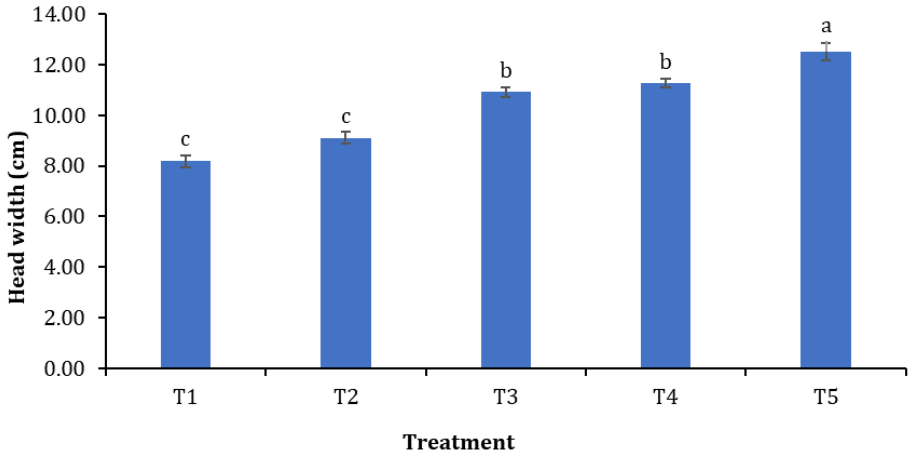

Figure 07. Effect of different biochar doses on head width.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Weight of head

In case of weight of head, it showed highly significant ($p < 0.01$) among the treatments. The maximum value was recorded in T_5 (765.00 g). The lowest weight with head was observed in T_1 (495.00 g) (Figure 08). The most important morphological trait is weight of head because it directly connected with yield performance of cabbage plant. There was a significant variation in the treatment of cabbage in case of weight of head in this study. Weight of head (495.00-765.00 g) was revealed in this experiment. A similar result was recorded by Radovich et al. (2004) and Tiffani et al. (2020). Promoting nutrient availability and uptake by plants along with maintaining the soil's moisture biochar contribute to increased biomass production and the increase in weight of the head (Yooyen et al., 2015).

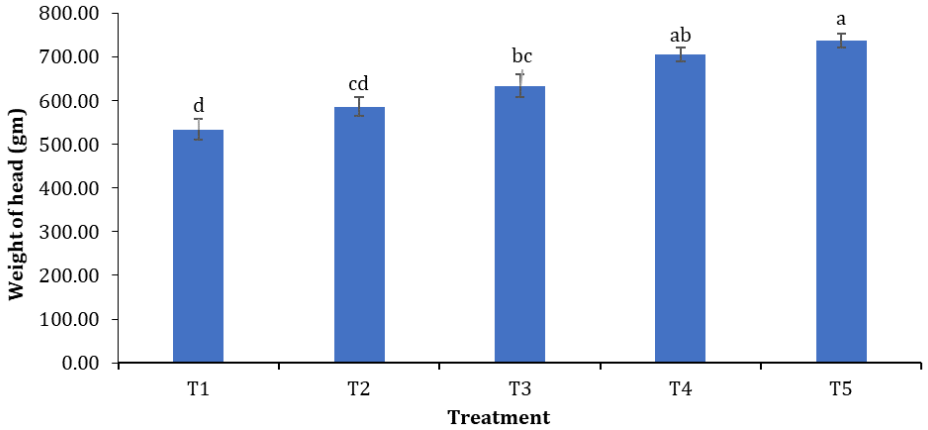

Figure 08. Effect of different biochar doses on weight of head.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c, d) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Dry weight of head

In case of dry weight of head, it showed highly significant ($p < 0.01$) among the treatment. The maximum value was recorded in T_5 (91.30 g) which is followed by T_4. The lowest weight with head was observed in T_1 (61.30 g). The T_2 and T_3 were also statistically grouped (Figure 09). Different studies reported that the dry weight of head of leafy vegetables statistically increased with the

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application of biochar (Coumar et al., 2016). Another study stated that biochar treatment in a cabbage increased plant height (Yooyen et al., 2015).

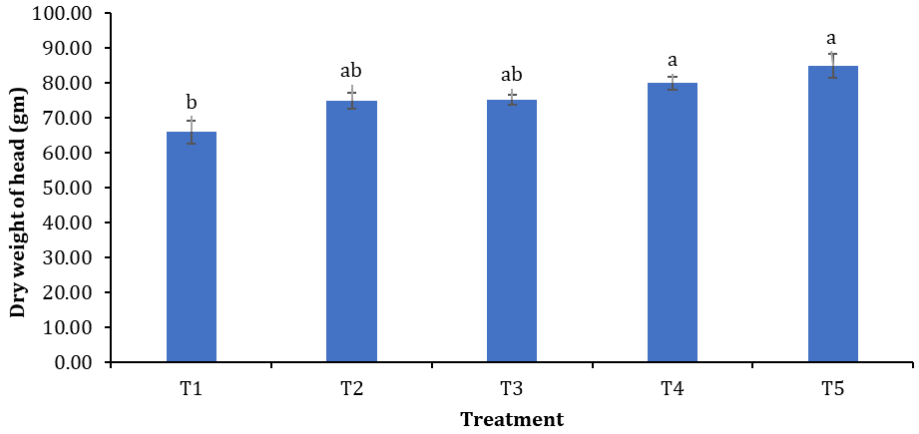

Figure 09. Effect of different biochar doses on dry weight of head.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Stump diameter

The highest head diameter was found in T_5 (2.20 cm). The lowest head diameter was observed in T_1 (2.00 cm) (Figure 10). Stump diameter is an important physiological character of cabbage since taste of cabbage depends on it. In our findings, stump diameter (2.00-2.20 cm) was revealed. According to Carter et al. (2012), biochar has a positive effect on the development and structural integrity of cabbage plants, potentially enhancing yield and quality. Also, different studies reported that the stump diameter of cabbage statistically increased with the application of biochar (Sarkar et al., 2021).

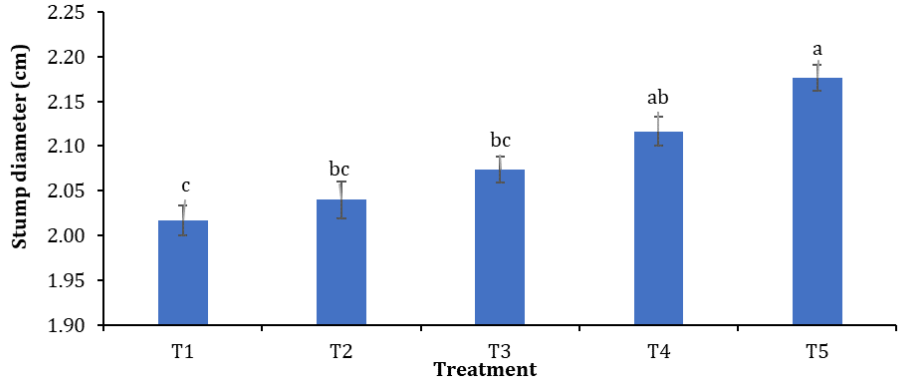

Figure 10. Effect of different biochar doses on stump diameter.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Stump length

In our study, it was shown that application of biochar has an impressive effect on increasing the length of stump per plant of cabbage. The length of stump per plant was increased with the doses of biochar. It showed highly significant ($p < 0.01$) among the treatments. The maximum value was recorded in T_5 (10.24 cm). The lowest value of stump length was observed in T_1 (6.92cm), followed by T_2 (Figure 11). According to the study cabbage stump length (6.92-10.24 cm) was observed. The result revealed that biochar improved stump length of cabbage plants. Different studies reported that the stump length of cabbage statistically increased with the application of biochar (Carter et al., 2013). Another study stated that biochar treatment in a cabbage increased head diameter (Hossain et al., 2010).

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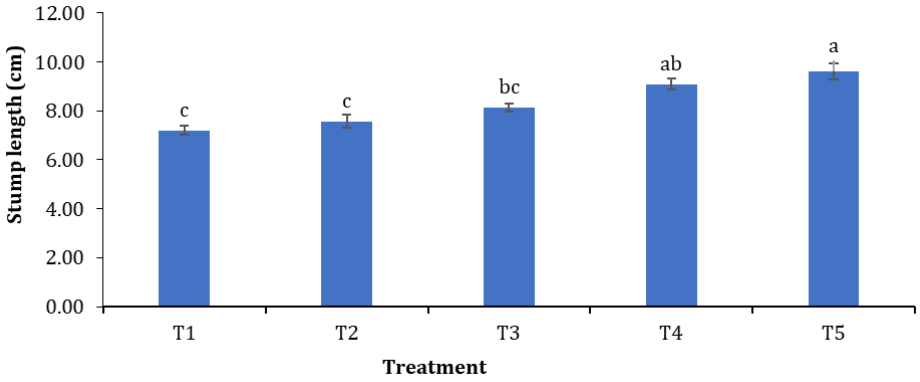

Figure 11. Effect of different biochar doses on stump length.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Root length

The study proved that biochar application influenced the root length of cabbage plant. The finding showed highly significant ($p < 0.01$) among the treatments. The maximum value was recorded in T_5 (21.00 cm). The lowest length of root was observed in T_1 (13.00 cm). The T_2 and T_3 were also statistically grouped (Figure 12). Root length of cabbage plays vital role in case of water and nutrient uptake. This study revealed the length of cabbage root in a range of 13 cm to 21 cm. Root length of cabbage statistically increased with the application of biochar which was similar to Hasan et al. (2012) findings. The enhanced root length observed with biochar application suggests improved below-ground development and capacity for nutrient acquisition. Biochar, with its porous structure and high surface area, creates a favourable rhizosphere environment, promoting root growth and proliferation (Bruun et al., 2014).

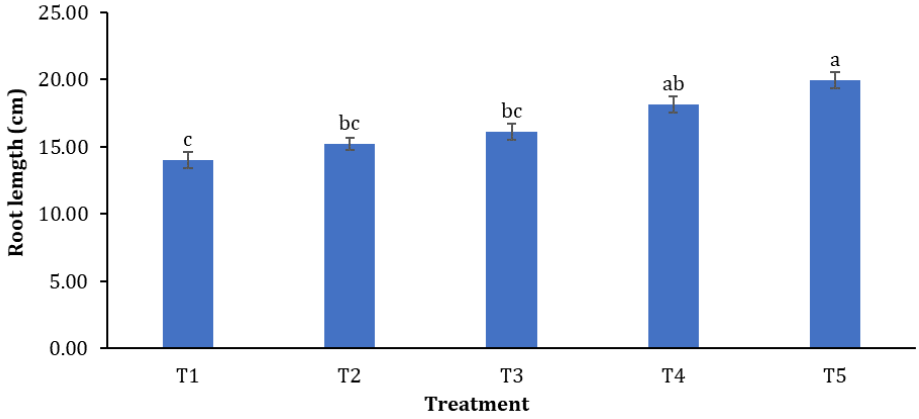

Figure 12. Effect of different biochar doses on root length.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b, c) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

Chlorophyll content

In saline soil, the effect of biochar application on the chlorophyll content of cabbage leaves was evaluated and found that they significantly increased the chlorophyll content. The result revealed that the chlorophyll content was increased with the increasing of biochar doses. The maximum value was recorded in T_5 (73.00) which is followed by T_4. The lowest weight with head was observed in T_1 (63.30). The T_2 and T_3 were also statistically grouped (Figure 13). Chlorophyll content (63.30-73.00 SPAD value) was revealed in this study. According to another study, chlorophyll content of cabbage statistically increased by the application of biochar which was similar to the result observed by an experiment (Chatterjee et al., 2010). As the improved water and nutrient-holding capacity of biochar have mitigated the adverse effects of salinity, allowing for better nutrient uptake and chlorophyll synthesis (Shashi et al., 2018).

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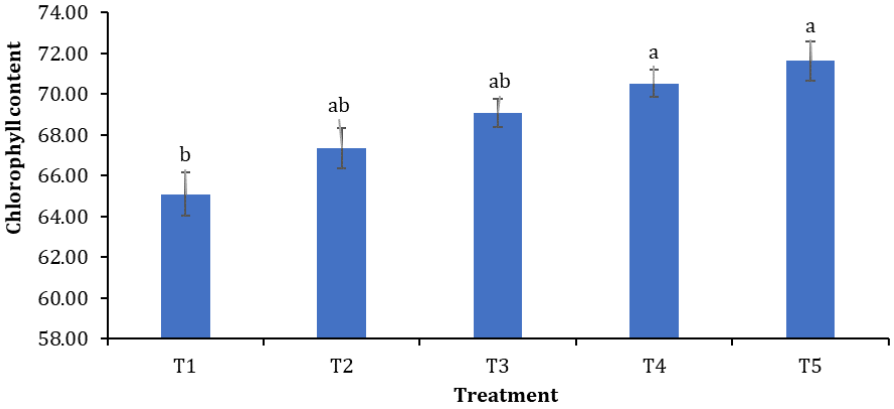

Figure 13. Effect of different biochar doses on chlorophyll content.

Vertical bars indicate standard error of mean against each variable. The values with different characters (a, b) indicate significant difference (*p≤0.05, **p≤0.01) over control. Here, T_1= control; T_2= 3.0 t ha^{-1}, T_3= 6.00 t ha^{-1}, T_4= 9.00 t ha^{-1}; T_5= 12.0 0 t ha^{-1} biochar.

IV. Conclusion

The results indicated that T_5 (12.0 t ha^{-1}) showed highest growth and yield than all other treatments. From our current findings, it can be stated Biochar has a significant influence on the growth and yield of cabbages, which indicates with increased doses of biochar the growth and yield in cabbages also increased. So, farmers might benefit through biochar application (at 12.0 t ha^{-1}) to boost cabbage yield, especially in the coastal region of Noakhali. As biochar application is influenced in cabbage production, other leafy vegetables can be grown with biochar application to evaluate their performance for ensuring sustainable vegetable production in coastal areas of Bangladesh.

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Conflict of Interest

The authors affirm that their publishing of this work does not present a conflict of interest.

V. References

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