Available online at http://www.sjbas.com.ng
Savanna Journal of Basic and Applied Sciences (June, 2025), 7(1): 1-1
P:ISSN 2705-3164 | E:ISSN 2695-2335

Impact of Biochar and Cowdung on Growth and Chlorophyll Content of Cucumber (*Cucumis sativus)*

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ABSTRACT

An experiment was conducted beside the central research laboratory of the University of Ilorin to study the effects of water hyacinth-derived biochar (WHB) and/or cow dung (CD) on the growth, yield, and chlorophyll content of *Cucumis sativus*. The treatment includes: 20 g WHB; 40 g WHB; 60 g WHB; 20 g CD; 40 g CD; 60 g CD; 10 g WHB+10 g CD; 20 g WHB+20 g CD; 30 g WHB+30 g CD. The treatments were laid out as a randomized complete block design with three replicates. Parameters assessed were plant height, leaf area, number of leaves, chlorophyll content (chlorophyll A, chlorophyll B, total chlorophyll, and carotenoids), fresh leaf weight, and dry leaf weight. Application of 60 g CD gave the highest leaf number (11.0 leaves/plants) and plant height (27.00 cm), and 40 g CD gave the highest leaf area (50.75 cm2). There is no significant difference among the treatments on the effects on chlorophyll content, but the highest value was recorded in 20 g CD (CHL A-1.31 mg/g; CHL B-0.84 mg/g; total CHL-21.58 mg/g; carotenoids: 4.24 mg/g). However, 60 g WHB produced the highest fresh leaf and dry weight (35.94 g and 13.47 g). It was concluded that using CD only has a promising effect on the plant growth and leaf area of *Cucumis sativus. At the same time,* the co-application of WHB and CD shows little or no enhancement, especially at high dosages when compared with the control and other treatment groups. 60 g WHB shows a promising effect on the fresh and dry weight of the *Cucumis sativus* leaf.

Keywords: Water Hyacinth Biochar, Cow Dung, *Cucumis sativus*, Chlorophyll Content.

INTRODUCTION

Recently, there has been a growing interest in using water hyacinth as a means of addressing its spread. Traditional methods of controlling this invasive plant, such as chemical, biological, and mechanical approaches, have had limited success. The challenge lies in eliminating water hyacinth (*Eichhornia crassipes*) but also in finding ways to transform it into something beneficial. This rapidly growing aquatic species often invades water bodies (Li *et al.*, 2018), and its potential use in biochar production is currently being explored (Chen *et al.*, 2010). A major concern is that poor management of water hyacinths could harm aquatic ecosystems and their inhabitants.

Converting this invasive species into biochar presents a promising solution to mitigate its harmful environmental effects (Masto *et al.*, 2013). Water hyacinth can spread rapidly, covering water surfaces and negatively impacting aquatic life, such as fish, by blocking light and depleting oxygen levels (Guerena *et al*., 2015). Additionally, using herbicides to control water hyacinths can increase greenhouse gas emissions through anaerobic decomposition. In contrast, converting this biomass into biochar offers a more sustainable alternative (Gunnarsson and Petersen, 2007; Guerena *et al*., 2015).

Biochar can be produced from natural biomass or organic waste, including food and agricultural residues, using various thermochemical methods, with pyrolysis being the most efficient and widely used (Antonakou *et al*., 2006). Research has shown that biochar derived from water hyacinth significantly enhances leaf mass (Cedeño *et al*., 2024). The effectiveness of biochar as a soil amendment depends on its specific physiochemical characteristics, which can vary based on the feedstock used (Seleiman *et al.*, 2020; Tomczyk *et al*., 2020). Biochar made from organic matter, such as manure, seaweed, and crop

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waste, tends to have a higher nutrient content compared to that produced from wood (Kavitha *et al*., 2018). Studies suggest that biochar can positively influence the physical, chemical, and biological properties of soil, thereby improving nutrient absorption and promoting plant growth (Blanco-Canqui, 2017).

Organic fertilisers are derived from plant and animal remains, such as manure, compost, green manure, straw, and other materials. These fertilisers help improve the physical, chemical, and biological properties of soil. Among the most commonly used organic fertilisers by farmers is cow dung (CD), which has been shown to enhance plant growth (Tanimu *et al*., 2013; Ra Himabadi *et al*., 2017). This growth boost is attributed to the essential nutrients present in cow manure, which meet the nutritional needs of plants. Research indicates that biochar can be used alongside organic fertilisers to improve soil structure, fertility, and crop yields (Agegnehu *et al*., 2016). Studies by Jabeen *et al*. (2018) have also found that organic manure can increase chlorophyll content in plant leaves.

However, there is limited information, especially in Nigeria, regarding the combined use of biochar from water hyacinth and cow dung as fertilisers for fruit vegetables like cucumber, despite the availability of these resources throughout the country. Noteworthy improvements in maize growth have been documented when vermicompost and biochar are applied together, in comparison to untreated plots that received only biochar (Doan *et al*., 2015). In the context of reducing waste and preventing harmful emissions, this study aims to investigate the effects of water hyacinth-derived biochar (WHB) and/or cow dung (CD) on the growth of Cucumis sativus. Thus, the major objective of the present study is to characterize biochar produced from water hyacinth, evaluate the effect of water hyacinth-derived biochar and/or cow dung on the physiological parameters of *Cucumis sativus,* evaluate the chlorophyll content of the leaf at harvest and determine the effect of water hyacinth derived biochar and/or cow dung on the fresh and dry weight of *Cucumis sativus* leaf

MATERIALS AND METHODS

Characterization of Water Hyacinth Biochar

A little portion of the Water Hyacinth Biochar (WHB) was taken to Rolab Research and Diagnostic Laboratory in Ibadan, Nigeria for characterization. Phase and crystal structure of the WHB was confirmed via X-ray diffraction (XRD) using an Ultima-III X-ray diffractometer (Rigaku, Japan) and Raman spectroscopy using a Reinshaw inVia confocal Raman microscope (England). The size and morphology were evaluated via scanning electron microscopy (SEM) using a FEI Magellan 400 XHR SEM (Hillsboro, OR). A Micromeritics 3Flex Surface and Catalyst 125 Analyzer (Norcross, GA) was employed to determine the Brunauer-Emmett-Teller (BET) surface area of the WHB.

Experimental Design and Analysis

The experiment was carried out using a Completely Randomized Design (CRD) with 3 replicates which was conducted at the side of Central Research Laboratory, University of Ilorin, Ilorin, Kwara state. The viable seed of *Cucumis sativus* was obtained from the University of Ilorin Herbarium. The water hyacinth was collected from the Aliara Asadam area of Ilorin, Kwara state, and sun-dried before processing it into biochar. The dried water hyacinth was pyrolysed using a local kiln technology which was set between the temperature of 550-600^{0}C. The biochar was then ground into powdery form and measured into different grams to be used. The cow dung was collected from Abbatoir center at the F-division area, Ilorin, sun-dried, and ground into a powdery form. The soil, cow dung, and/or WHB were homogenously mixed and left for two weeks before planting on it. After planting, measurements of parameters were taken at 2, 6, and 8WAP. The data collected were plant height, number of leaves, leaf length, and leaf breadth. Fresh and dry weights of the leaves were also determined. The treatment used is 20 g WHB; 40 g WHB; 60 g WHB; 20 g CD; 40 g CD; 60 g CD; 10 g WHB+ 10 g CD; 20 g WHB+ 20 g CD; 30 g WHB+ 30 g CD. The data collected were subjected to one-way Analysis of Variance (ANOVA) and because the experiment was conducted using a completely randomized design, the means were separated using the Duncan multiple range test (DMRT) at a 5% significance level using SPSS 20. The determination of chlorophyll concentration was done as proposed by Makeen *et al.* (2007)**.**

Chl a (mg g-1) = $[(12.7 \times A_{663}) - (2.6 \times A_{645})] \times$ ml acetone / mg leaf tissue ------- Eq. 1

Chl b (mg g-1) = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times$ ml acetone / mg leaf tissue ------ Eq. 2

C x+c = 1000 A_{470} – 1.90Chl a – 63.14 Chl b / 214, (x = xanthophylls and c = carotenes) --- Eq. 3

Total Chl = 20.3A645 + 8.02A663 ---------- Eq. 4

RESULTS

Application of 40 g WHB only had the highest significant effects on the plant height (6.95 cm) of *Cucumis sativus* ($P \leq 0.05$) when compared with other treatments used in this study at 2WAP, followed by 20

g CD (6.90 cm) and then 20 g WHB+20 g CD (6.85 cm) while the shortest plant height was seen in 40 g CD (6.15 cm). The highest leaf length, leaf breadth and leaf area were observed in treatment with 20 g CD (2.35 cm, 3.25 cm and 5.55 cm^2 respectively). The lowest leaf length was measured in 20 g WHB+20 g CD with value 1.60 cm. similarly, the lowest leaf breadth and leaf area was observed in 60 g WHB (1.95 cm and 2.65 cm^2 respectively) (Table 1). At 6WAP (Table 2), the highest cucumber plant height and leaf number was measured in treatment 60 g CD (22.40 cm and 8.50) at (P≤0.05) while the lowest height was observed in 20 g WHB (17.20 cm). The largest leaf area was produced from 40 g CD (48.70 cm^2), this is followed by 60 g WHB (38.20cm^2). At 8WAP, the highest number of leaves (11.0 leaves/plant) was produced by 60 g CD, 10.0 from 20 g CD, 40 g CD and 30 g WHB+30 g CD while the lowest was observed in the control with 8.0 leaves /plants). There is no significant difference among the number of leaves in all treatment at P≤0.05. The highest plant height (27.0 cm) was produced by in plant that received 60 g CD treatment, followed by 40 g CD (23.8 cm) and 20 g CD (22.15 cm) while the lowest (17.65 cm) was observed in 20 g WHB treatment which is significantly different from t the 60 g CD treated plants (Table 3).

There is no significant difference in the chlorophyll content of *Cucumis sativus* leaf at P≤0.05 Table 4). The highest CHL content (CHL A, CHL B, TOTAL CHL and CARTD were observed in the plant that received the 20 g CD (1.31 mg/g, 0.84 mg/g, 21.58mg/g and 4.24 mg/g). the lowest CHL A, CHL B and TOTAL CHL values were observed in 40 g WHB treated plants with 0.83 mg/g, 0.40 mg/g and 12.34 mg/g respectively. For carotenoid, the lowest was observed in 30 g WHC+30 g CD with 2.56 mg/g. Furthermore, the effects of the treatment on the leaf fresh and dry weight of cucumber (Table 5). Treatment with 60 g WHB had the highest fresh and dry weight (35.94 g and 13.47 g), the highest value is followed by 40 g CD (32.85 g) and then 60g CD (27.19 g) for fresh weight while the dry weight followed 60 g CD (4.35 g) and then 20 g WHB+20 g CD (4.32 g). for fresh weight the lowest was observed in the control (18.30 g) while the lowest dry weight was produced in 20 g CD (2.27 g) treated plant.

Table 1: Effects of WHB and/or CD on the morphological character of *Cucumis sativus* at 2WAP

TREATMENT	NOL	PH (cm)	LL (cm)	LB (cm)	LA (cm)
Control	3.00±0.00^{a}	6.5±0.70	2.27±0.03	3.20±0.00	5.45±0.07^{ab}
20 g WHB	3.00±0.00^{a}	6.25±0.63	1.90±0.28	2.20±1.27	3.25±02.33^{ab}
40 g WHB	3.00±0.00^{a}	6.95±0.07	1.90±0.42	2.45±0.49	3.43±0.98^{ab}
60 g WHB	2.00±0.00^{b}	6.55±0.91	1.70±0.56	1.95±0.63	2.65±1.62^{b}
20 g CD	2.00±0.00^{b}	6.90±0.70	2.35±0.21	3.25±0.70	5.55±0.35^{a}
40 g CD	3.00±0.00^{a}	6.15±0.21	1.67±0.45	2.90±0.14	3.75±1.06^{ab}
60 g CD	2.50±0.00^{ab}	6.75±0.77	2.25±0.70	3.00±0.42	5.05±0.91^{ab}
10 g WHB+10 g CD	2.00±0.00^{b}	6.50±0.98	2.00±0.28	3.05±0.35	4.60±1.13^{ab}
20 g WHB+20 g CD	3.00±0.00^{a}	6.85±0.21	1.60±0.56	2.85±0.70	3.40±1.13^{ab}
30 g WHB+30 g CD	2.50±0.00^{ab}	6.25±0.21	2.10±0.14	3.05±0.35	4.80±0.84^{ab}

Values carrying the same letter (s) within the same column are not significantly different at p<0.05 using ANOVA and DMRT WHB=Water Hyacinth Biochar; CD=Cow Dung; PH= Plant Height; NOL=Number of Leaves; LL=Leaf length; LB=Leaf Breadth; LA=Leaf Area; cm=centimeters

Table 2: Effects of WHB and/or CD on the morphological character of *Cucumis sativus* at 6-WAP

TREATMENT	NOL	PH (cm)	LL (cm)	LB (cm)	LA (cm)
Control	8.00±1.41	17.60±4.68^{b}	5.90±0.70^{b}	6.50±0.98^{b}	28.95±7.84^{b}
20 g WHB	8.50±0.07	17.20±1.13^{b}	5.95±0.70^{b}	5.95±0.70^{b}	29.90±4.80^{b}
40 g WHB	7.50±0.07	17.85±0.91^{ab}	5.90±0.14^{b}	7.30±0.14^{ab}	32.15±1.62^{b}
60 g WHB	8.50±0.07	18.15±0.91^{ab}	6.45±0.21^{ab}	7.90±0.84^{ab}	38.20±5.37^{ab}
20 g CD	8.00±0.00	19.75±1.06^{ab}	6.65±0.63^{ab}	7.10±0.14^{ab}	35.30±2.68^{ab}
40 g CD	8.50±0.70	19.30±1.69^{ab}	7.45±0.35^{a}	8.70±1.13^{a}	48.70±8.62^{a}
60 g CD	8.50±0.70	22.40±0.56^{a}	6.70±0.98^{ab}	6.70±0.98^{b}	34.00±9.89^{ab}
10 g WHB+10 g CD	7.50±0.70	18.00±2.12^{ab}	5.95±0.63^{b}	6.60±1.13^{b}	29.70±8.20^{b}
20 g WHB+20 g CD	8.00±0.00	17.45±0.63^{b}	5.50±0.00^{b}	6.15±0.77^{b}	25.35±3.18^{b}
30 g WHB+30 g CD	8.00±0.00	17.50±0.70^{b}	5.55±0.07^{b}	6.05±0.77^{b}	25.15±2.89^{b}

Values carrying the same letter (s) within the same column are not significantly different at p<0.05 using ANOVA and DMRT

WHB=Water Hyacinth Biochar; CD=Cow Dung; PH= Plant Height; NOL=Number of Leaves; LL=Leaf length; LB=Leaf Breadth; LA=Leaf Area; cm=centimeters

Table 3: Effects of WHB and/or CD on the morphological character of *Cucumis sativus* at 8-WAP

TREATMENT	NOL	PH (cm)	LL (cm)	LB (cm)	LA (cm)
Control	8.50±3.53	19.60±6.92^{b}	6.15±1.20^{bc}	6.95±0.91^{ab}	32.45±10.53^{b}
20 g WHB	9.00±1.42	17.65±0.49^{b}	7.05±0.70^{ab}	7.20±1.41^{ab}	38.05±7.84^{ab}
40 g WHB	9.50±0.07	20.65±0.21^{ab}	6.30±0.28^{bc}	7.60±0.56^{ab}	35.95±4.31^{ab}
60 g WHB	9.50±0.07	21.65±2.47^{ab}	6.90±0.56^{ab}	7.95±0.77^{ab}	41.25±7.42^{ab}
20 g CD	10.00±0.00	22.15±0.07^{ab}	6.20±0.14^{bc}	7.05±0.70^{ab}	32.75±1.06^{b}
40 g CD	10.00±1.41	23.80±3.39^{ab}	7.80±0.56^{a}	8.65±0.91^{a}	50.75±8.98^{a}
60 g CD	11.00±0.00	27.00±2.82^{ab}	6.25±0.70^{bc}	6.45±0.35^{b}	30.15±1.34^{b}
10 g WHB+10 g CD	9.50±0.70	21.85±3.74^{ab}	5.95±0.63^{bc}	6.80±0.98^{ab}	30.55±7.70^{b}
20 g WHB+20 g CD	9.00±0.00	20.25±0.63^{ab}	5.32±0.10^{c}	6.35±0.63^{b}	25.25±2.05^{b}
30 g WHB+30 g CD	10.00±0.00	20.85±0.21^{ab}	5.85±0.21^{bc}	6.35±0.91^{b}	27.90±5.09^{b}

Values carrying the same letter (s) within the same column are not significantly different at $p<0.05$ using ANOVA and DMRT WHB=Water Hyacinth Biochar; CD=Cow Dung; PH= Plant Height; NOL=Number of Leaves; LL=Leaf length; LB=Leaf Breadth; LA=Leaf Area; cm=centimeters

Table 4: Effects of WHB and/or CD on the chlorophyll content of *Cucumis sativus*

TREATMENT	CHL A (mg/g)	CHL B (mg/g)	TOTAL CHL (mg/g)	CARTD (mg/g)
Control	1.13±0.34	0.60±0.24	17.34±5.92	3.61±1.42
20 g WHB	1.07±0.04	0.74±0.28	18.22±3.29	3.17±0.03
40 g WHB	0.83±0.01	0.40±0.01	12.34±0.06	2.88±0.33
60 g WHB	1.12±0.36	0.56±0.21	16.92±5.76	3.02±0.88
20 g CD	1.31±0.67	0.84±0.52	21.58±12.07	4.24±0.54
40 g CD	1.02±1.11	0.55±0.06	15.78±1.79	3.48±0.01
60 g CD	1.22±0.18	0.61±0.11	18.42±2.92	3.52±0.74
10 g WHB+10 g CD	1.03±0.23	0.53±0.16	15.62±4.00	3.32±1.40
20 g WHB+20 g CD	0.84±0.09	0.43±0.31	12.75±1.22	2.68±0.01
30 g WHB+30 g CD	0.85±0.40	0.43±0.31	12.90±0.78	2.56±0.35

Values carrying the same letter (s) within the same column are not significantly different at $p<0.05$ using ANOVA and DMRT WHB=Water Hyacinth Biochar; CD=Cow Dung

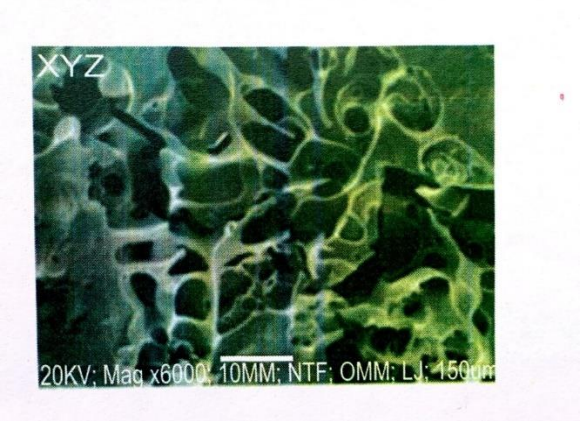
Table 5: Effects of WHB and/or CD on the fresh and dry weight of *Cucumis sativus* leaves

Treatment	Fresh Weight	Dry Weight
Control	18.30±6.39	3.17±0.72
20 g WHB	19.50±5.70	2.72±0.72
40 g WHB	22.17±4.13	3.24±0.56
60 g WHB	35.94±18.75	13.47±14.84
20 g CD	17.03±1.11	2.27±0.31
40 g CD	32.85±20.65	3.08±0.06
60 g CD	27.19±7.33	4.35±0.95
10 g WHB+10 g CD	16.91±0.48	2.45±0.51
20 g WHB+20 g CD	20.99±6.34	4.32±3.16
30 g WHB+30 g CD	20.05±7.87	2.85±0.72

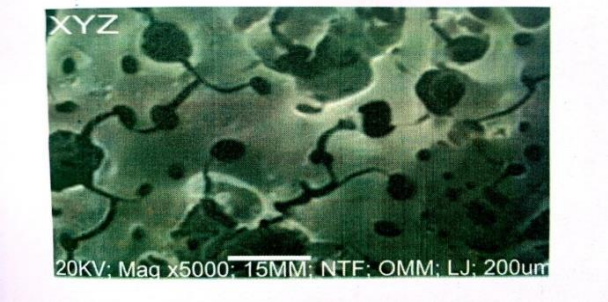
Values carrying the same letter (s) within the same column are not significantly different at $p<0.05$ using ANOVA and DMRT WHB=Water Hyacinth Biochar; CD=Cow Dung



1. **SEM at 50µm wavelength**



2. **SEM at 150µm wavelength**



3. **SEM at 200µm wavelength**

Figure 1: Photomicrograph of Water Hyacinth Biochar (WHB) SEM

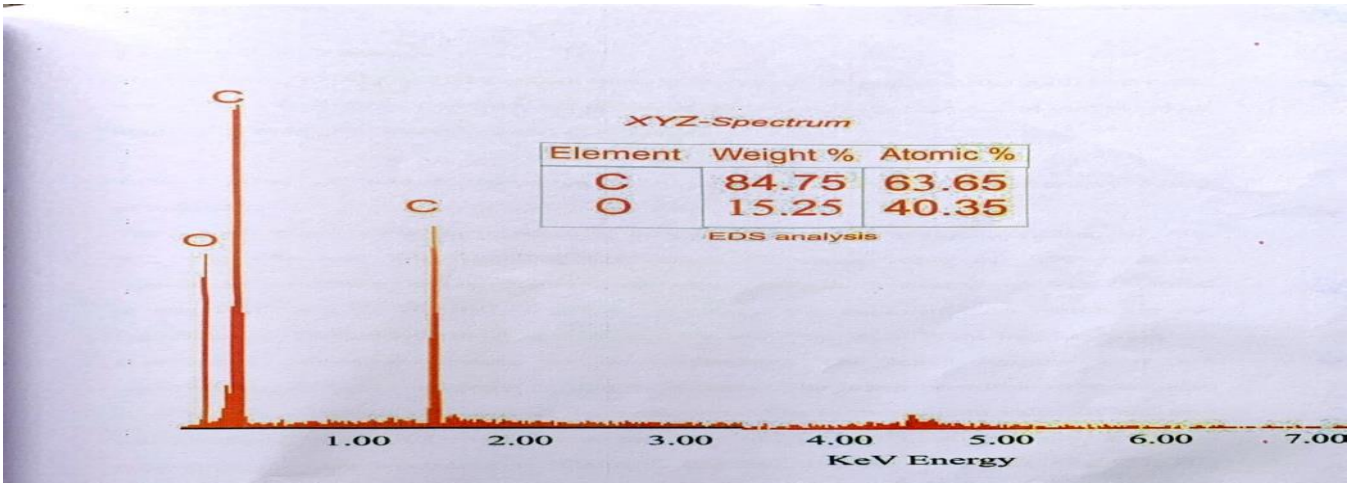


Plate 2: EDX Analysis Result of Water Hyacinth Biochar (WHB)

DISCUSSION

Combined application of WHB + CD, WHB only, and CD only had a significant effect on the plant when compared with control at the early stage of planting but this was contrary at maturity. It was observed that the plant that has the sole application of CD treatment has the highest vegetative growth (60 g CD), several leaves (60 g CD), and leaf area (40 g CD) and this is consistent with the findings of Mojeremane (2015) who reported that the application of organic fertilizer greatly enhanced growth, development, and yield performance of rape in terms of plant height, leaf length, leaf breadth, leaf area, and leaf number. Similarly, Musdalifah *et al*. (2013) found that cow dung is a good fertilizing material that can be used to maintain soil fertility status and improve crop

production. The decrease in plant growth that was observed in 20 g WHB+20 g CD in this study is not in line with the findings of Agegnehu *et al*. (2016) who reported that compost application with biochar has shown a more prominent role in improving crop performance. However, this may be due to factors like repulsion between the two amendments which have effectively enhanced the growth of the cucumber plant.

The leaf is the factory of food synthesis in the plants, which contributes toward final grain yield. If the leaves stay green for a long duration, the process of photosynthesis will take place smoothly and this will ensure better grain yields. The highest chlorophyll content was obtained from the 20 g CD plants. 40 g WHB induced the lowest chlorophyll content in this investigation. The results obtained from this study were in agreement with what Jabeen *et al*. (2018) reported. They reported that organic manure increases chlorophyll content in plant leaves of spinach beet (*Beta vulgaris* var. *bengalensis* L.). Singh *et al*. (2014) and Narkhede *et al*. (2011) also reported an improvement in the chlorophyll content of chili leaves with increasing manure application rates. More chlorophyll content in leaves might be due to macro and micro nutrients supplied by organic manure particularly nitrogen which is an important constituent of chlorophyll (Jabeen *et al*., 2018)

Fresh and dry weight of leaves

The plant that received the 60 g WHB shows significant effects on the fresh and dry weight of the cucumber leaves. The lowest dry weight was reported in the plant treated with 10 g WHB+10g CD. The finding of this study is in line with the investigation of Cedeño *et al*. (2024) who reported that the leaf mass fraction of plants treated with biochar increased when compared with the control.

Conclusion and Recommendation

The use of CD only has a promising effect on the plant growth and leaf area of Cucumis sativus while the co-application of both WHB and CD shows little or no enhancement, especially at high dosage when compared with the control and other treatment groups. 60 g WHB shows a promising effect on the fresh and dry weight of the leaf. The use of CD only or with another type of agricultural waste is recommended.

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