



INTERNATIONAL JOURNAL OF INNOVATION AND INDUSTRIAL REVOLUTION (IJIREV) www.ijirev.com



ASSESSING THE EFFECT OF BIOCHAR ADDITION IN GROWING MEDIUM ON PLANT GROWTH PERFORMANCE OF BOK CHOY (*Brasicca Rappa* Subsp. *Chinensis*) USING SUSTAINABLE SOILLESS AGRICULTURE SYSTEM

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Article Info:

Article history:

Received date: 26.03.2023 Revised date: 30.04.2023 Accepted date: 31.05.2023 Published date: 27.06.2023

To cite this document:

Ali, S. N., Aziz, A. A., Kasim, N. N., Tajuddin, S. A., Omar, N. F., & Hirun, N. N. (2023). Assessing The Effect Of Biochar Addition In Growing Medium

Abstract:

COVID-19 pandemic has revealed several extreme weather conditions which caused damage to nature and decrease in crop production. This has resulted to increased demand for food and raise concerns related to food security such as issues of hunger and poverty. Soilless agriculture has become a preferable approach to curb this issue due to benefits such as preventing soil-related disease and deforestation. Soilless agriculture uses biomass like cocopeat as growing medium. Previously, biochar has shown a better result in plant growth as compared to cocopeat media alone, but unsuitable concentration could be ineffective or induce plant disease. Hence, in this study, the influence of biochar added into the growing medium was investigated on the growth of Bok Choy (*Brassica rapa* subsp. *Chinensis*) using the sustainable Zero-Energy Soilless Agriculture (ZESA) System. Three treatments of growing medium T1



On Plant Growth Performance Of Bok Choy (*Brasicca Rappa* Subsp. *Chinensis*) Using Sustainable Soilless Agriculture System. *International Journal of Innovation and Industrial Revolution*, 5 (13), 104-118.

DOI: 10.35631/ IJIREV.513010

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(100% cocopeat), T2 (82% cocopeat, 18% biochar), and T3 (63% cocopeat, 37% biochar), were conducted using Completely Randomized Design (CRD) to design the plot treatment. The plant growth parameters of height, number of leaves, and fresh weight were observed for 28 days at 7 days intervals. The results indicated that T1 exhibited the highest number of new leaves (3 – 4 leaves per week), plant height (4.7 cm) and fresh weight (53.87 g), followed by T2 and T3. Analysis of variance (ANOVA) revealed that biochar addition has significant difference in means treatments of 0.029 between T1 and T2. Interestingly, T3 has the potential as a good growing medium as analysis of leaves after harvesting showed that this growing medium promotes N uptake of the plant as compared to other treatments.

Keywords:

Biochar; Bok Choy; Food Security; ZESA System

Introduction

The sustainable development goals (SDGs) 2025 recognize that eliminating poverty requires strategies that promote economic growth and address social, climate, and environmental issues such as food crisis, pollution, and global warming (Bhakta et al., 2021). The recent COVID-19 pandemic had revealed a record-breaking heatwave, rainfall, and flooding worldwide, which caused damage to nature and food insecurity (Rasul, 2021). As a result, food security has become a huge issue for the community when there is insufficient crop production and high demand for food which leads to problems such as increasing food prices, hunger, and poverty. Agriculture is widely recognized as an important sector to curb the problems of food security.

The soilless agriculture system is a technique of growing plants without using soil, which could overcome problems arising from the use of soil, such as soil disease. This study was utilizing a Zero-Energy Soilless Agriculture (ZESA) system, an innovation for agriculture practices that is beneficial in terms of small spaces with less water uptake and nutrient monitoring (Kasim et al., 2021). As this system is using cocopeat as the soilless growing medium, the problems related to agriculture using soil could be avoided.

Nutrient such as nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) are essential macronutrient for the horticultural plant to increase plant growth performance (Xu et al., 2021). It was reported that renewable waste is an excellent feature to be applied as a nutrient source by converting it into biochar. This is because biochar is a stable carbon with high N and K that is essential to the plant (Rawat et al., 2020). Nevertheless, unsuitable concentrations of biochar reported to be ineffective or inducing plant diseases (Silva et al., 2015).

In this study, the effect of adding biochar into the soilless growing medium on plant growth performance was investigated. The plant chosen is Bok Choy (*Brassica rapa* subsp. *Chinensis*), a short cycle horticultural plant. The objectives of this study are: i) to investigate the effect of biochar addition into growing medium on Bok Choy's plant height, number of leaves, and fresh weight produced using ZESA System; and ii) to determine the effect of biochar addition in growing medium on N uptake of Bok Choy by elemental analysis of the leaves after harvesting.



Literature Review

There are three points discussed in Literature Review, which are soilless cultivation in agriculture, Bok choy and biochar as nutrient source in plant growth.

Soilless Cultivation in Agriculture

Soilless cultivation is the process of growing plants without using soil as a rooting medium. The main benefit of soilless cultivation is preventing plant growth from issues like soil-borne pests, soil fertility, and greenhouse problems (Fussy & Papenbrock, 2022). It was also reported that soilless cultivation helps enhancing fresh produce's yield, quality, and safety by adapting open or closed systems with the use of biomass as a rooting medium (Tzortzakis et al., 2020). Furthermore, the practice of soilless cultivation offers help with land availability and water supply for agricultural crops, dwindling, population growth, and soil degradation.

ZESA System

Zero-Energy Soilless Agriculture (ZESA) is a system that combines hydroponic and fertigation techniques. It is a hybrid of plant growth system that employs a porous soilless growing medium of biomass and self-nutrient intake via a piping system, making it suitable for fast-growing plants, especially the horticultural plants (Kasim et al., 2021). As a result, nutrient deficiencies below the root area can be reduced, thereby overcoming the issues associated with poor nutrient supply and waste of energy consumption. Additionally, this system can be used in sheltered and small-space areas while requiring less watering and nutrient monitoring than the hydroponic system. Figure 1 shows a diagram of ZESA system used in this study.

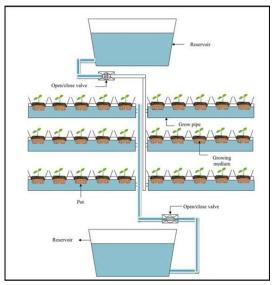


Figure 1: ZESA system

Source: Kasim et al. (2021)

Bok Choy

Brassica rapa subsp. *Chinensis*, or common name – Bok Choy or Pak Choi is one of Chinese cabbage from a genus of Brassica family (Zhang et al., 2016). It is high in nutrition, such as vitamin C, E, fibres, and phenolic compounds (PC). PC is crucial in plant because of its high antioxidant activity and vigorous plant growth against pests and environmental stress (Du et al., 2022). The substantial number of phytochemicals and antioxidant potential make the Brassica family a preferable plant for nutritional and pharmaceutical applications (Nawaz et al., 2018). Figure 2 shows Bok Choy plant.

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Figure 2: Bok Choy Plant

Source: Galeri Pak Choi Hidroponik (n.d.)

Nutrients for Development of Bok Choy

Nutrients are an integral part of the development of Bok Choy. The nitrogen N supply to the *Brassica* plant is of vital importance to ensure plant growth. It is one of the most critical limiting factors in cultivation and plays a role in many metabolic and biosynthetic pathways (Veazie et al., 2020). N deficiency in plants reduces photosynthesis and affects yield production by inhibiting the synthesis of chlorophyll and proteins (Anas et al., 2020; Wang et al., 2019). Additionally, the function of N has effect on the greenness of Bok Choy because N improves plant pigmentation (Anas et al., 2020). Another research also has identified that when the plant obtain an adequate supply of N, the chlorophyll rate will increase and improve plant leaf pigmentation (Fathi & Branch, 2022). In other work, the plant treated with N reported to has higher plant height than the untreated plant (Razaq et al., 2017).

Based on Truong (2017), N and K treatments were found to have better overall plant growth than the control. Xu et al. (2020) claimed that the moderate supply of K will enhance the development of plant root. When plants get sufficient level of K, the plants will increase the protein production as this nutrient helps in reading the genetic code that regulates all growth processes. The plants would also has less potential to get infections due to the crucial character of K in plant's metabolism which help to endure under biotic or abiotic stress (Truong, 2017).

Other than that, the addition of sulfur (S) in the cultivation of Bok Choy is crucial for synthesizing amino acids and cellular components such as thiol and secondary sulfur compounds to protect plants against pests and soil stress (Zenda et al., 2021). The deficiency amount of S will decrease the crops yield and the quality of plant will reduce as a result of chlorosis in young leaves (Narayan et al., 2022). Table 1 summarizes functions of nutrients for development of Bok Choy.



Table 1: Functions of Nutrients for Development of Bok Choy					
Nutrients	Functions	Reference			
Nitrogen (N)	The most critical limiting factors in cultivation and	Veazie et al.			
	plays a role in many metabolic and biosynthetic pathways	(2020)			
	Deficiency in plants reduces photosynthesis and	Anas et al. (2020)			
	affects yield production by inhibiting the synthesis	Wang et al. (2019)			
	of chlorophyll and proteins				
	Improves plant pigmentation	Anas et al. (2020)			
	Increase plant height	Razaq et al. (2017)			
Potassium (K)	Moderate supply will enhance the development of	Xu et al. (2020)			
	plant root				
	Help to endure under biotic or abiotic stress (less	Truong (2017)			
	potential to get infections)	-			
Sulfur (S)	Crucial for synthesizing amino acids and cellular	Zenda et al. (2021)			
	components to protect plants against pests and soil				
	stress				
	Deficient amount will decrease the crops yield and	Narayan et al.			
	the quality of plant due to chlorosis in young leaves	(2022)			

Biochar as a Nutrient Source for Plant Growth

Biochar is a charcoal-like substance made by burning organic-based material from biomass in a controlled process such as gasification or pyrolysis at 300 - 600 °C under the exclusion of oxygen. Among the raw materials used to produce biochar are forestry by-products, organic material waste, and agriculture residue (Yaashikaa et al., 2020). During pyrolysis, the organic material from these biomass sources is converted into biochar, a stable form of carbon. Every particular type of biochar contains C, H, O, N and other minerals such as Si, Al, Fe, P, and S in their composition at different levels (Xiao et al., 2018; Guo et al., 2019). In terms of physical attributes, biochar displays an excellent feature for the potential application, such as highly porous micro-morphology, high absorption, and an extensive surface area (Oni et al., 2019).

In general, biochar has been reported to have several advantages in agricultural application. Guo (2020) claimed that applying biochar to soils without chemical fertilization improves plant growth and agricultural output, which is primarily due to minor nutrients such as P, Mg, and K in the ash from biochar components. Previous work by Frenkel et al. (2017) reported that biochar has giving positive impact on plant growth compared with peat media only in the presence of more than 25% of concentration (v:v). Concentration of biochar was found to affect plants with less than 1% weight will cause plant disease while more than 3% by weight induced plant disease or considered as ineffective (Silva et al., 2015). They also reported on the usage of 0.4 - 0.8% mischantus biochar on the growth of *Brassica rapa* plant. The result obtained, however, signaled the negative impact of this biochar as the presence of *Plasmodiophora brassica* pathogen was observed.

This study will also use the plant of the similar family with lower doses of store-bought biochar were used for growing the plant in a soilless cocopeat growing medium. This is because additional additives at lower doses have been proven able to improve plant performance against pathogens (Poveda et al., 2021). In addition, this approach also helps biochar to make up a significant amount of the soilless media and could potentially substitute peat on a substantial



scale (Frenkel et al., 2017). Aroloye (2020) also reported that using biomass in a soilless medium will reduce the amount of carbon dioxide (CO₂) and sulfur dioxide (SO₂) pollutants, which make biochar an environmentally friendly nutrient source. Table 2 summarizes the role of biochar as nutrient source of plant growth.

Table 2: Role of Biochar as Nutrient Source for Plant Growth					
Author(s)	Findings				
Guo (2020)	Biochar to soils without chemical fertilization improves plant growth and agricultural output				
Frenkel et al. (2017)	Biochar has giving positive impact on plant growth compared with peat media only (more than 25% v/v of concentration)				
	Biochar to make up a significant amount of the soilless media and could potentially substitute peat on a substantial scale				
Silva et al. (2015)	Less than 1% weight will cause plant disease, more than 3% by weight induced plant disease or considered as ineffective				
、 <i>,</i>	0.4 - 0.8% mischantus biochar on the growth of <i>Brassica rapa</i> plant - presence of <i>Plasmodiophora brassica</i> pathogen.				
Poveda et al. (2021)	Lower doses of biochar have been proven able to improve plant performance against pathogens				

Nonetheless, the usage of mangrove biochar in soilless agriculture systems is not being investigated yet. In addition, the effect of biochar addition into growing medium on N uptake of plant in this sustainable system is not being extensively explored. Therefore, it is attractive to investigate the potential of mangrove biochar, as an addition in soilless agriculture, particularly the ZESA System.

Methodology

Materials

Growing Medium

The growing medium of cocopeat was purchased from Abi Agro in Perlis, while the biochar was purchased from local store. Both cocopeat and biochar were removed from impurities and dried, then stored in an airtight container for further use. Table 3 shows properties of the growing medium.

Table 3: Properties of Cocopeat and Biochar				
Growing medium	Cocopeat	Biochar		
Moisture content (%)	61.60	7.88		
Elemental Analysis (%)				
С	42.04	28.42		
Н	6.05	3.99		
Ν	3.94	3.38		
S	0.14	0.27		
0*	47.83	63.94		

*Obtained by difference



High moisture content of cocopeat implies its ability to retain high amount of water due to its hydrophilic features which can attract water in large amount (Ilahi & Ahmad, 2017). This could enhance the wettability of the plant by absorbing water flow from the ZESA System's pot, thereby helps in water efficiency of the Bok Choy that can support the plant development (Shahmihaizan et al., 2021). Cocopeat and biochar has comparable content of N and S, which are important for plant growth.

Fertilizer Solution

XY fertilizer of brand "Joe Jalil Concept" was bought from Abi Agro in Perlis. The fertilizer solution was prepared according to the instructions stated on the container's label.

Bok Choy Plants

As fertilizer is only suitable to be introduced following the seedling becoming a sapling, this study was using Bok Choy sapling as a plant of interest. Bok choy plants were bought from "Kebun Hidroponik Abi Agro Perlis" and their growth was observed throughout the duration of the study.

Experimental Setup

The experiment was carried out in the nursery at Unit Ladang, UiTM Arau, Cawangan Perlis with coordination of $6^{\circ}27'07'.9"$ N 100°16'49.9" E and have exposed temperature estimated from 24 °C to 29.2 °C in Arau, Perlis.

Plants Process Setup

The Bok Choy plants were planted by measuring the size of the root ball of the sapling to estimate the length of the wide pit and deep soil amendment to dig out. The plants were then placed in each of the holes at the center pit and filled with cocopeat.

ZESA System Setup

Figure 3 shows a ZESA system used for observing Bok Choy's growth performance in an open system. It consists of an open and close valve, reservoir tank, grow pipe, pot, and growing medium (cocopeat). The effect of biochar on plant growth was investigated using this system with adaptation of previous work by Azhar (2022).



Figure 3: ZESA Setup

Growth Performance of Bok Choy

For growth performance, the first-time interval was recorded after the saplings have been transferred to their individual pots. The plant growth was observed for 28 days at 7 days

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intervals. Plant height and number of leaves of Bok Choy during each interval and following harvesting were measured accordingly. Data of fresh weight was only obtained following harvesting.

Design of Experiment

CRD for ZESA System

Three replicates (3 x 6 = 18 plots) were arranged by using three treatments with 2 individuals. About three-quarters of a medium-sized pot was filled up with the treatment of biochar and cocopeat. Three medium treatments were employed with T1 represents cocopeat without biochar as control, T2 represents 82% cocopeat with 18% biochar, and T3 represents 63% cocopeat with 37% biochar. The CRD in this study required 18 treatments and the analysis of variance (ANOVA); one-way analysis. One-way ANOVA was used to make a comparison between the two or more independent variables that influenced the plant performance.

Plot Treatment Design

The location treatments in the completely randomized block design are visualized in Figure 4.

T				
	T1R2I1	T2R4I1	T2R3I1	
	T2R1I1	T3R2I1	T3R3I1	
6 ×	T3R1I1	T2R2I2	T2R3I2	
ŝ	T2R1I2	T3R2I2	T3R3I2	
	T3R1I2	T2R3I1	T1R1I1	
	T2R2I1	T3R3I1	T3R4I1	
<u>3×6</u>				

Figure 4: Randomized Block Design for Each Treatment

Statistical Analysis of Data

Data from three replicates were collected and tabulated in a proper format. The Statistical Package of the Social Science (SPSS) software was used to calculate standard deviation, sum of squares, and means using ANOVA for CRD.

Analysis of N Content in Leaves

The PerkinElmer 2400 CHNS Analyzer was used to analyze the amount of N in the leaves sample.

Results and Discussion

Growth Performance of Bok Choy

The effect of different treatments (T1, T2, T3) of growing medium using cocopeat and mangrove biochar on the growth performance of Bok Choy (*Brassica rapa subsp. Chinensis*)



was observed. The duration of observation has been limited to 28 days (four weeks) due to several issues, such as bad weather, presence of parasite and animal interference, and problem of piping system.

Effect of Different Treatments on Plant Height

Figure 5 shows the effect of different treatments on Bok Choy's plant height. The first and second week of cultivation showed a decrease height of plants in the range of -54.7% due to the unpredictable weather in Arau, Perlis. During this time, the temperature has fallen to a minimum of 23 °C with heavy rain and a maximum of 32 °C during hot weather. The unbalanced site field and leaking of pipe also affected the plant height as an adequate amount of nutrients could not be supplied, making the plant wilted and reduced in size.

During weeks 3 to 4, the weather has improved, and the plant's height increase by 3.64% indicates a good growth performance of Bok Choy. The plant height at the final week of harvested time for plant with treatment of T3 (4.6) has displayed a significant development as the plant height has comparable height with that of T1(4.7) and higher than T2 (3.9). This finding shows treatment with 36% of biochar has the potential to become additional growing medium with similar effect with cocopeat on plant height effect.

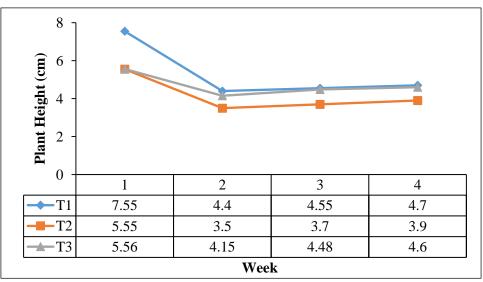


Figure 5: Effect of Different Treatments on Plant Height of Bok Choy

Effect of Different Treatments on Number of Leaves

Figure 6 shows the number of leaves increasing over the week of cultivation, indicating all treatments are having a positive effect in producing new leaves. The sapling of Bok choy contains 8 to 11 leaves at the beginning of cultivation and grows 2-4 leaves every week. Plants from T1 grew by 3-4 leaves weekly; while plants from T2 and T3 grew 2-3 leaves every week. However, during the observation, some of the plant's leaves were eaten by parasites such as snails and black caterpillars, which caused leaves' damage and stunt the plants' growth. The physical properties of the plants (green colour) also attract the parasite to consume the plants. According to the Harrington (n.d.), Bok Choy is one of the plant was usually affected due to the pest and plant disease.



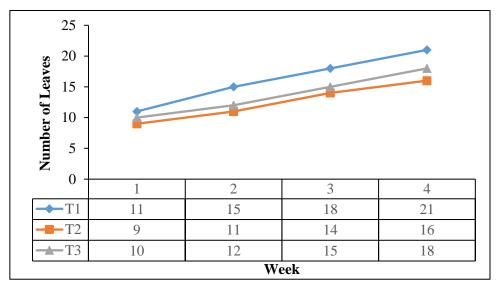


Figure 6: Effect of Different Treatments on Number of Leaves of Bok Choy

Effect of Different Treatments on Fresh Weight of Bok Choy

Figure 7 shows the effect of different treatment on fresh weight of Bok Choy. In general, the fresh weight of the Bok choy was affected by the height of the plant and the number of leaves. As a result, plants from T1 with higher number of leaves have the highest fresh weight at 53.87 g, compared to T3 (42.03 g) and T2 (38.1 g). Detailed observation showed that the fresh weight of plants from T2 has been affected due to its stunted growth. This is because two pots from T2 were highly affected by parasites and the plants' leaves have been eaten. Since the number of plant leaves and the height plant has a parallel relationship with the fresh weight (Zhang et al., 2017), fresh weight of the plants from T2 decreased significantly as compared to other treatments.

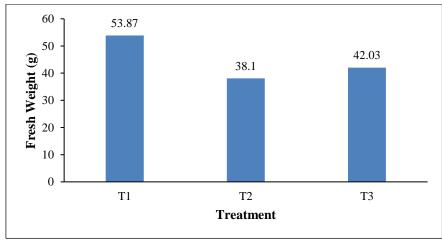


Figure 7: Effect of Different Treatments on Fresh Weight of Bok Choy

Statistical Analysis of Data

One-Way ANOVA using SPSS has been used for the statistical analysis of data. Table 4 tabulated the summary of result in plant height and the number of leaves for every treatment in four weeks. The result for plants fresh weight was not included in ANOVA because the fresh weight was measured only once during the harvest time.

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International Journal of Innovation and Industrial Revolution EISSN: 2637-0972

Volume 5 Issue 13 (June	2023) PP. 104-118
DOI 10.356	31/IJIREV.513010

		able 4: ANOVA of Plant Height and Number of Leaves				
		Sum of Squares	Df	Mean Square	F	Sig.
DL	Between Treatments	9.742	2	4.871	4.110	0.021
Plant Height	Within Treatments	81.771	69	1.185		
	Total	91.512	71			
Number of Leaves	Between Treatments	76.563	2	38.281	3.104	0.051
	Within	850.938	69	12.332		
	Total	927.500	71			

Table 4: ANOVA of Plant Height and Number of Leaves

From the data analysis, the result for number of leaves was accepted (p > 0.05) with value of 0.051. Meanwhile, result for plant height also showed significant differences between the treatment with the p-value of 0.021 (p < 0.05) (Grabowski, 2016).

Since the null hypothesis, H_0 is rejected, Post Hoc Test such as Tukey Test then being performed.

Table 5 compares multiple treatments after an initial analysis has been performed. Also referred to as multiple comparison tests or pairwise comparison tests, it is used to determine which specific treatments are significantly different from each other. From the analysis, it was observed that there is a significant difference between the means treatments of T1 and T2 (0.029). This result shows that T1 was better than T2 but T2 is lower than T3 which indicates growing medium of cocopeat alone is better than growing medium of cocopeat and biochar for the growth of Bok choy using ZESA system. While, adding 38% biochar into the cocopeat is a more suitable concentration of growing medium for Bok choy growth as compared to adding 18% of biochar into the cocopeat.

Table 5: Post Hoc Test							
			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence	
Dependent	(I) Treatment	(J) Treatment					
Variable						Interval	
variable						Lower	Upper
						Bound	Bound
Plant	T1	T2	1.128	0.430	0.029	0.097	2.159
Height	11	T3	0.603	0.430	0.346	-0.428	1.634
	T2	T3	-0.525	0.272	0.138	-1.177	0.126
Number of	T1	T2	3.281	1.388	0.054	0438	6.606
Leaves	11	T3	1.969	1.388	0.337	-1.356	5.295
	T2	T3	-1.313	0.878	0.300	-3.415	0.790



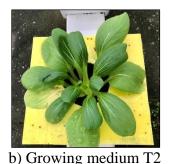
Analysis of N Content in Leaves

The N uptake of leaves in growing medium has a different effect on plant properties. It was found that the amount of biochar addition into the growing medium has affected the N level of leaves. Leaves from growing medium T1 and T2 contain 0% N, while leaves from growing medium T3 was 2.52% N, revealing that leaves from T3 have the most significant N content compared to leaves of other treatments. This finding indicates the possible effect of biochar content in growing medium that promotes N uptake in plants.

Figure 8 shows the effect of different treatments on leaves pigmentation. Visual observation revealed that growing medium of T3, with a total of 37% of biochar gives a good pigment to the leaves. This showed the level of N uptake to the plants gives a significant impact on the leaves color (Yu et al., 2020). On the contrary, the leaves grown under T1 and T2 were slightly pale and yellow. A visual characteristic of the nitrogen deficit in T1 (Figure 7 a) and T2 (Figure 7 b) after harvested were displayed. This symptom has appeared from week 2 following the cultivation process, and continuously becomes pale and yellow until harvesting time. Figure 8 c) shows the overall leaves of the Bok Choy in T3 were in good condition and greener than others.



a) Growing medium T1





c) Growing Medium T3

Figure 8: Effect of Different Treatments on Leaves Pigmentation

Conclusions

From this result, it can be deduced that biochar has no significant impact on plant growth performance of Bok Choy. However, this result reveals important information such as that the additional of biochar in unsuitable concentration could be ineffective or induce plant disease. Observation on plant using growing medium of T2 shows that combination of cocopeat and biochar at this concentration is not suitable since this plant attracted parasite such as snail and causing the fresh weight, number of new leaves and plant height to be lower than plants from other treatments. Growing medium of T3 has comparable effect with that of T1, indicating the potential of biochar in substituting cocopeat as soilless growing medium. The analysis of N content in leaves revealed the role of biochar in providing nutritional components to the plant. This can be seen as no N was detected in the plant leaves from T1 and T2. Meanwhile the presence of N was detected in T3, indicating T3 has potential to increase N uptake of the plant and prevent N deficiency of Bok choy. Future work includes nutrient analysis of the plant to assess the impact of adding biochar into the growing medium on the chemical compositions of the plant produced.



Acknowledgement

The authors would like to extend the gratitude to Universiti Teknologi MARA Cawangan Perlis for providing support in terms of space for plant cultivation and other facilities to complete this study.

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