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Growth, Phytochemicals, and Antioxidants of Bok Choy Plants (*Brassica rapa* L.) Grown in Plant Factory, Greenhouse Hydroponics, and Soil

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Abstract—Bok choy (*Brassica rapa* L.) is a plant with high economic value and is often consumed by people but has problems in its cultivation in the field. Land conversion results in many issues in the farming of this plant, hence plant factory and hydroponics system are a viable alternative which can affect growth and phytochemical contents which affect antioxidant activity. Hence, research is needed to discover the difference in growth, phytochemical content, and antioxidant activity. This research compared the growth of bok choy plants grown in plant factory, hydroponics in greenhouse, and soil. Extracts from the leaves of bok choy produced in each agriculture system were then analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) and antioxidant activity analysis was done using DPPH (2,2-diphenyl-1-picrylhydrazyl) test. Bok choy plants grown hydroponically in a greenhouse showed the greates growth and greatest number of phytochemicals, followed by bokchoys grown in plant factory, and bokchoys grown in soil. Additionally, bokchoys grown hydroponically in a greenhouse had highest number of phytochemicals detected, however bokchoys grown in a plant factory showed the strongest antioxidant activity.

Keywords: Antioxidant, Bok choy, Greenhouse, Hydroponics, andPhytochemicals.

I. INTRODUCTION

Bok choy (*Brassica rapa* L.) is a plant in the *Brassicaceae* family. Bok choy is a plant with high economic value and fast production time [1]and because of its high nutritional value on terms of vitamins and minerals which can help reduce risks of illnesses [2]. However, issues in bok choy production have recently cropped up due to limitations on land and climate change which have resulted in economic loss for farmers and have influenced the supply of bok choy plants themselves. One of the solutions to this problem is hydroponic-based agriculture which is a form agriculture which does not require soil and instead uses water as a medium [3]. Hydroponic systems do not require large amounts of land and space as it is possible to use verticulture. Additionally, hydroponic systems can be installed indoors in places such as a greenhouse to protect crops from pest attacks and irregular weather. A derivate of hydroponic agriculture is a plant factory, which is a facility which can increase production yields and grow crops anywhere and anytime regardless of the environmental factors in the location[4]. However, change in agriculture methods may result in the change of phytochemical contents of the plant, as seen with a research conducted by Bantis[5] where different light spectrums used in a plant factory resulted in different phytochemical content. Phytochemicals are secondary metabolites which possess physiological functions to plants and also to the animals and humans who consume these plants. Phytochemical content is heavily linked to the antoxidative qualities of a plant, as some phytochemicals are powerful antioxidants. Additionally, the nutrient solution used in hydroponics play an important role in the formation of phytochemicals and antioxidants [6]. Different agricultural systems and different environmental factors have a profound impact on the growth, phytochemical content, and antioxidant activity of bok choy plants hence this research aimed to discover which agricultural metode was the best for growing bok choy as well as the differences in phytochemical content and antioxidant activity.

II. EXPERIMENTAL DETAILS

This research was conducted between January and April 2023, with the bok choy plants being grown in the Faculty of Agriculture’s Experimental Garden located in Bali, Indonesia with the coordinates; 8°42'22.8"S 115°12'58.3"E. Samples were then analyzed in the Genetic and Molecular Biology Laboratory and the Unified Laboratory of the Mathematics and Natural Sciences Faculty, whereas the GC-MS analysis was conducted in the Forensic Laboratory of the Denpasar’s Police Force.

2.1 Bok Choy Growth

The bok choy seeds used in this research were of the Nauli F1 variety. The seeds were first spread over damp, rockwool media (2,5cm in thickness) on a seeding tray and covered with a black plastic bag to stimulate germination. After 24 hours, the plastic bag was removed and the seeds were left to sow for 14 days. After 14 days, the seeds were transferred to a plant factory facility, a hydroponic system installed in a greenhouse, and polybags filled with approximately 1kg soil media from Pubotan. In both the plant factory facility and greenhouse hydroponic system, the nutrient solution used was based on Suryantini[7] and kept at a static concentration of 1000ppm. The plant factory facility used artifical lighting through light-emitting diodes (LED) coloured red, blue, and white (RBW) kept on for 18 hours a day with 6 hours off. Lighting in the greenhouse as well as the outdoors for the bok choy grown in soil, was dependant on the natural sunlight in the research location which was 12 hours of daytime and 12 hours of nighttime. No additional fertilizers and pesticides were used in this research.

2.2 Growth Analysis

Bok choy plants from each agriculture method was harvested at the age of 54 days after sowing. Five samples were chosen randomly from each agriculture method, with the paramaters of observation being plant height, number of leaves, stem diameter, chlorophyll content of leaves, fresh weight, and dry weight. The data from the samples of each agriculture method was then averaged and compared. Comparation was done using One-way ANOVA with LSD Post-Hoc Test at a difference of 0,05.

2.3 Sample Extraction

Samples for GC-MS analysis and antioxidant analysis were obtained from the dried bok choy plants. The bok choy plants had first been dried using sunlight for 2 days under shade and then using a dry oven with a constant temperature of 45,0ºC for 5 days. After measuring the dry weight of each sample, the leaves were then cut into small pieces and then ground into bok choy simplicia. The simplicias were then macerated using 96% ethanol by pouring the aforementioned ethanol into beaker containing the simplicia until it was submerged. The solution was left for 3 days under dark conditions with the occasional stirring. After 3 days, the solution was strained and the remaining solute macerated once more. This process was repeated 3 times until the extract was coloured a deep, opaque, green. The macerate was then evaporated using a rotary evaporator at a temperature of 35ºC until a thick, paste-like extract was obtained.

2.4 GC-MS Analysis

The bok choy extracts of the three agriculture methods were then diluted with ethanol 96% 20 times their initial concentration. After dilution, each of the samples were then centrifuged for 3 minutes and the supernatant liquid was injected into the HP-5MS UI column of the GC-MS machine. This GC-MS analysis used nitrogen (N_2) as the carrier gas. The oven was first heated to 50ºC and the temperature increased 7ºC per minute until the final temperature reached 300ºC. Once the final temperature was reached, it was kept constant for 5 minutes and then cooled down until 110ºC when 1µl of the sample was injected into the column. The chromatogram results were then compared to the Wiley database and further analysed using PubChem.

2.5 Antioxidant Activity Test

The antioxidant activity of the samples were tested using *2,2-diphenyl-1-picrylhydrazil*(DPPH). The bok choy extracts were first diluted using ethanol to various concentrations between 100-1000ppm. 5ml of the diluted extract was pipetted into a round-bottom flask and 5ml of DPPH solution was added into the flask and mixed with ethanol until it reached the 25ml line. The solution was then left for 20-30 minutes and then the absorbancy was measured using a UV-spectrophotometer with a maximum wavelength of 517nm. This process was repeated with the other extracts.

III. RESULTS AND DISCUSSION

3.1 Results

No.	Bok Choy Plants Source	Average Height (cm)	Average Number of Leaves	Average Stem Diameter (cm)
1.	Plant Factory	11,6b	21,0b	1,0b
2.	Hydroponic in a Greenhouse	13,8c	17,6b	1,28c
3.	Soil Media	9,2a	4,8a	0,43a

Table 1. One-Way ANOVA Results of Height, Number of Leaves, and Stem Diameter. Different letter following a number indicates a significant difference at 0,05 value.

One-way ANOVA anaylysis showed that there was a significant difference between the bok choys grown at 3 different locations at the 0,05 difference. Tabel 1 shows that bok choys grown hydroponically in a greenhouse had the tallest plants on average with a height of 13,8cm, followed by bok choys grown in plant factory at 11,6cm, and finally bok choys grown conventionally in soil at 9,2cm. The bok choys with the highest average amount of leaves were the ones grown in the plant factory with a final-leaf count of 21 leaves, followed by bok choys grown hydroponically in a greenhouse with 17,6 leaves, and bok choys grown in soil media with 4,8 leaves. Bok choys grown hydroponically in a greenhouse had the heighest average stem diameter at 1,28cm, followed by bok choys grown in plant factory with an average stem diameter of 1,0cm, and finally by bok choys in soil media with an average stem diameter of 0,43cm.

No.	Bok Choy Plants Source	Average Chlorophyll Content
1.	Plant Factory	51,24b
2.	Hydroponic in a Greenhouse	46,82b
3.	Soil Media	26,5a

Table 2. One-Way ANOVA Result of Chlorophyll content. Different letter following a number indicates a significant difference at 0,05 value.

On the other hand, Tabel 2 shows that bok choys grown in plant factory had the highest amount of chlorophyll with a 51,24 SPAD units, followed by bok choys grown hydroponically in a greenhouse at 46,82 SPAD units, and bok choys grown in soil media at 26,5 SPAD units.

No.	Bok Choy Plants Source	Average Fresh Weight (g)	Average Dry Weight (g)
1.	Plant Factory	253,6b	14,8b
2.	Hydroponic in a Greenhouse	280,4b	20,0c
3.	Soil Media	8,0a	0,29a

Table 3. One-Way ANOVA Results of Fresh and Dry Weight. Different letter following a number indicates a significant difference at 0,05 value.

Based on Tabel 3, bok choys grown hydroponically in a greenhouse had the largest fresh weight and dry weight at 253,6g and 14,8g respectively, followed by bok choys grown in the soil at 8,0g and 0,29g. There was a significant difference between all three agriculutural methods in the parameters of plant height, stem diameter, and dry weight. On the other hand, there was a significant difference between bok choys grown hydroponically in a greenhouse and plant factory compared to bok choys grown in the soil in the observed parameters of number of leaves, chlorophyll, and fresh weight.

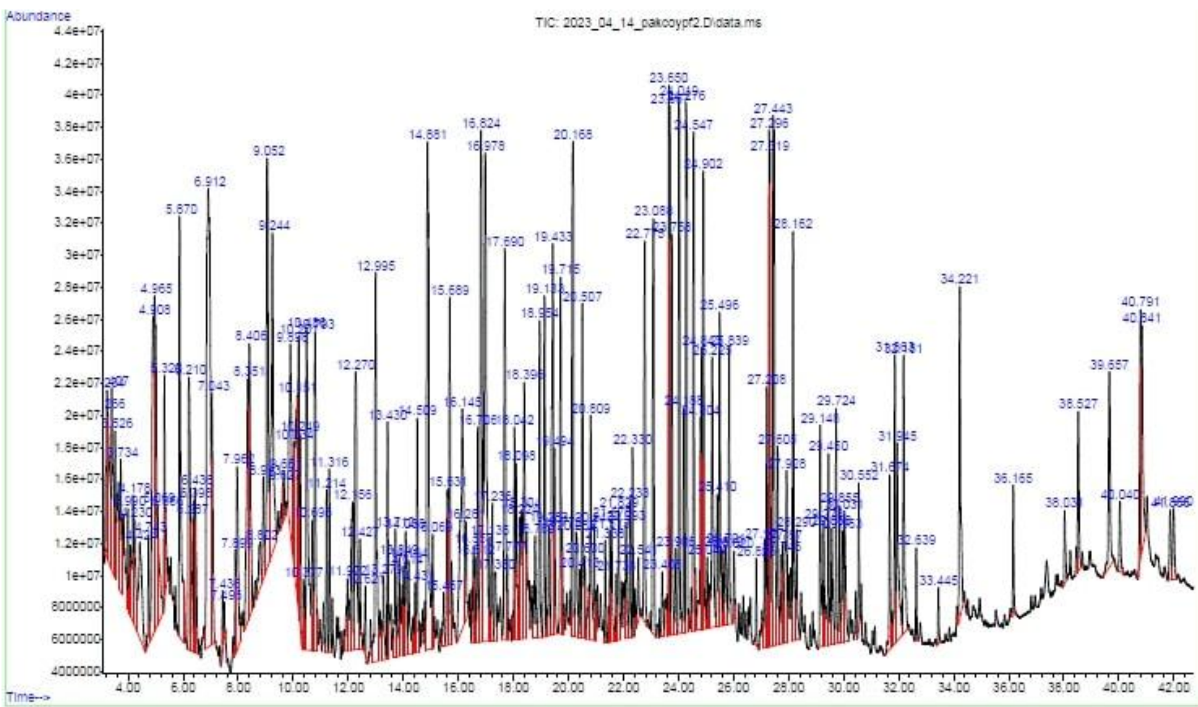

Figure 1. GC-MS Chromatogram of Bok Choys Grown in Plant Factory

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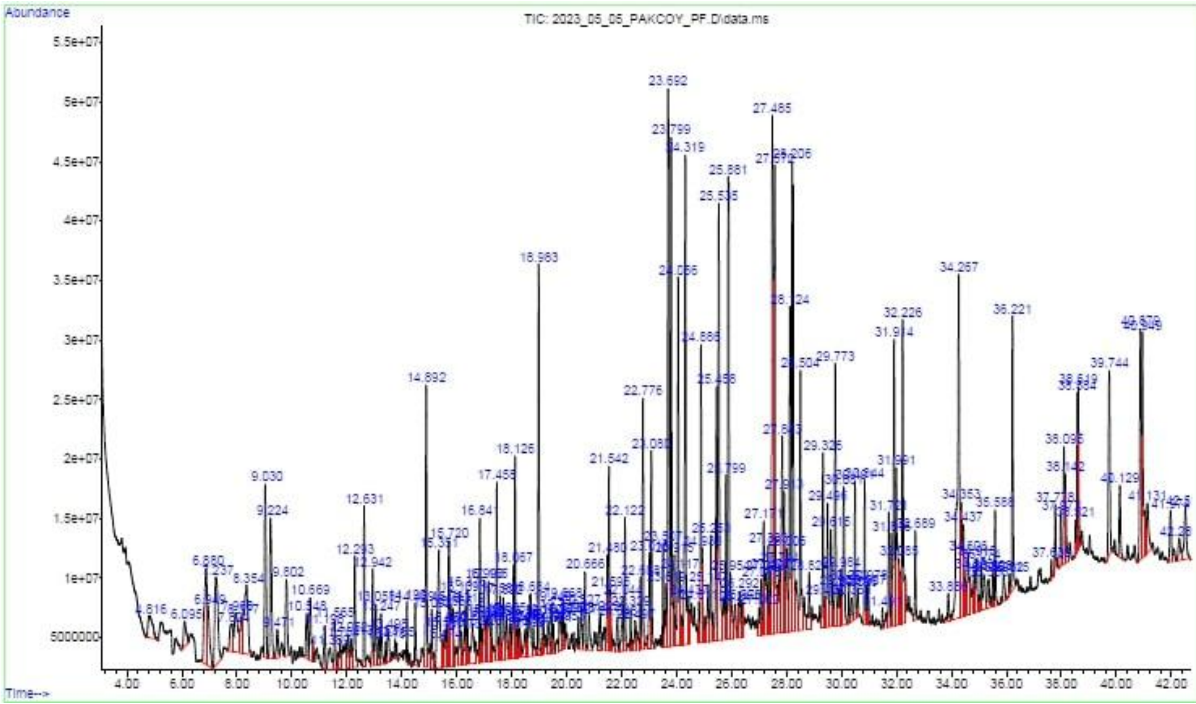

Figure 2. GC-MS Chromatogram of Bok Choys Grown Hydroponically in a Greenhouse

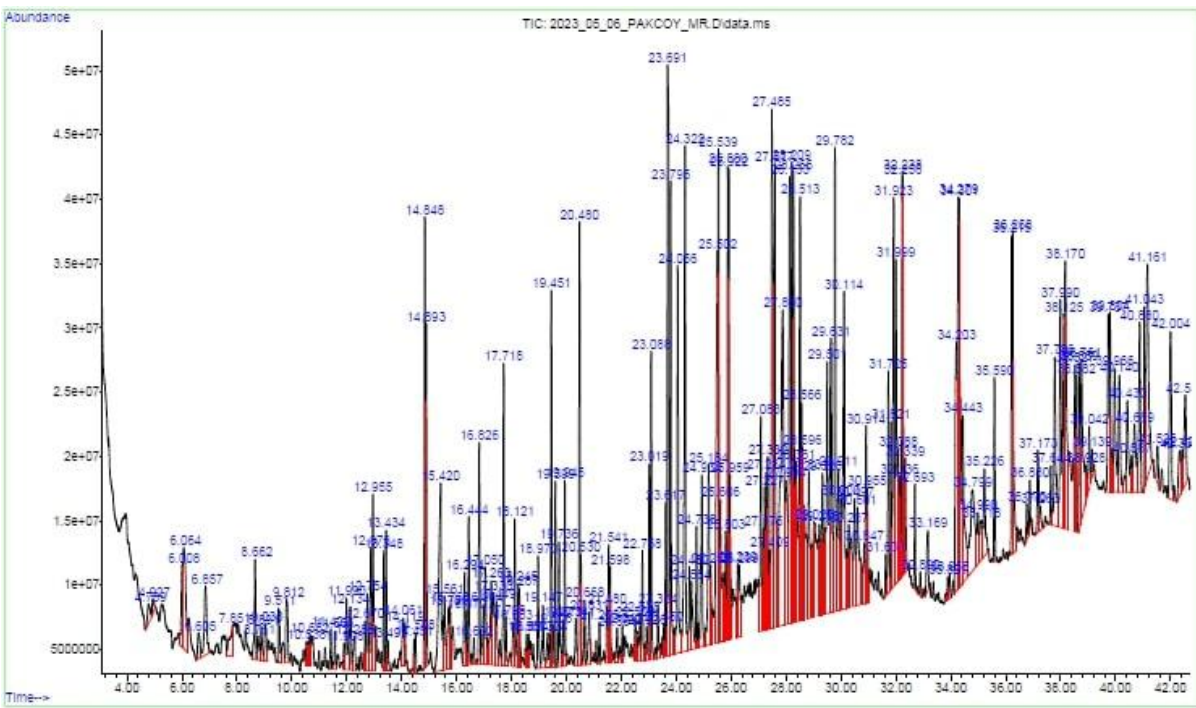

Figure 3. GC-MS Chromatogram of Bok Choys Grown Conventionally in Soil Media

Based on Fig.1, Fig.2, and Fig.3, GC-MS result of bok choys extracted from the three methods of agriculture had neophytadiene ($C_{20}H_{38}$) as the phytochemical with the highest *peak* which holds anti-inflammatory properties [8].

Name of Phytochemical	**Molecule Formula**	**Retention Time (RT)**	**Area Under Curve (AUC)**	**Function of Phytochemical**
Cyclopentylamine	$C_5H_{11}N$	6.912	3,58	Anti-biofilm and anti-adhesion towards microbes [9].
3'-Methoxyacetophenone	$C_9H_{10}O_2$	14.881	2,31	Antioxidant [10]
Phytol	$C_{20}H_{40}O$	27.443	2.15	Anti-inflammation, anti-cancer, and anti-fungal [11]
4,7-Dimethylbicyclo[3.2.1]oct-e-en-6-one	$C_{10}H_{14}O$	20.168	1.75	Anti-fungal [12]
Butylamine	$C_4H_{11}N$	9.052	1.58	Anti-inflammation dan antioxidant [13]
Phenethylamine	$C_8H_{11}N$	10.486	1.4	Antibacterial [14]
4-Vinylphenol	C_8H_8O	12.995	1.39	Anti-inflammation, used in treatment of arthritis and asthma [15]
Cyclohexanol	$C_6H_{12}O$	5.87	1.39	Anti-depressant and used in anesthesia [16]
2,6-Dimethoxy-4-vinylphenol	$C_{10}H_{12}O_3$	19.433	1.31	Antioxidant and antimicrobial and anti-inflammation [17,18].
2-Piperidinone	C_5H_9NO	12.27	1.31	Anti-fungal [14]

Table 4. Phytochemical Content of Bok Choys grown in Plant Factory

Based on Table 4, bok choys grown in plant factory had cyclopentylamine ($C_5H_{11}N$) as the phytochemical with the largest area under curve (AUC) at 3,58% which possesses anti-biofilm and anti-adhesion properties towards microbes [9].

Name of Phytochemical	**Molecule Formula**	**Retention Time (RT)**	**Area Under Curve (AUC)**	**Function of Phytochemical**
Phytol	$C_{20}H_{40}O$	27.485	3.3	Anti-inflammation, anti-cancer, and anti-fungal [11]
Neophytadiene	$C_{20}H_{38}$	23.692	3.06	Anti-inflammation [8]
Nonanoic acid	$C_9H_{18}O_2$	34.267	3.04	Anti-fungal [19]
Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	25.535	2.39	Anti-fungal dan anti-bacterial [14]
Ethyl palmitate	$C_{18}H_{36}O_2$	25.881	2.33	Anti-virus [20]
Actinidiolide, dihydro	$C_{11}H_{16}O_2$	18.983	1.55	Anti-algae [21]
2-Palmitoyglycerol	$C_{19}H_{38}O_4$	32.226	1.45	Anti-bacteria [17]
Ethyl linoleate	$C_{20}H_{36}O_2$	28.124	1.44	Anti-inflammation [17]
Butyl 9,12,15-octadecatrienoate	$C_{22}H_{38}O_2$	31.914	1.44	Anti-inflammation, antioxidant, dan anti-tumor [22]
Campesterol	$C_{28}H_{48}O$	40.879	1.42	Component in

				estrogenhormonal control drugs [22]

Table 5. Phytochemical Content of Bok Choys Grown Hydroponically in a Greenhouse

Table 5 shows that bok choys grown hydroponically in greenhouse and soil had phytol ($C_{20}H_{40}O$) as the highest AUC at 3,30%. Phytol is a chemical with anti-inflammatory, anti-cancer, and anti-microbial properties [1]. There were also a few differences in the contents of phytochemicals between bok choys grown in a plant factory and grown in a greenhouse.

Name of Phytochemical	**Molecule Formula**	**Retention Time (RT)**	**Area Under Curve (AUC)**	**Function of Phytochemical**
Phytol	$C_{20}H_{40}O$	27.49	2.15	Anti-inflammation, anti-cancer, and anti-fungal [11]
Neophytadiene	$C_{20}H_{38}$	23.69	2.08	Anti-inflammation [8]
Cyclononane	C_9H_{18}	29.78	1.72	Antioxidant [23]
Ethyl linolenate	$C_{20}H_{36}O_2$	28.13	1.66	Anti-inflammation [19]
Ethyl linolenate	$C_{20}H_{34}O_2$	28.21	1.5	Herbicidal [24]
Palmitic acid	$C_{16}H_{32}O_2$	25.5	1.48	Antioxidant and nematicide [14]
Nonanoic acid	$C_9H_{18}O_2$	34.3	1.46	Anti-fungal [19]
Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	25.54	1.44	Anti-fungal and anti-bacterial [14]
1,5-dimethyl-2-(2-dimethylaminoethyl)-3-morpholinomethylindole	$C_{19}H_{29}N_3O$	29.63	1.41	Antioxidant, anti-inflammation, and anti-microbes [17]
Ethyl stearate	$C_{20}H_{40}O_2$	28.51	1.39	Used in soap and cosmetics [25]

Table 6. Phytochemical Content of Bok Choys Grown Conventionally in Soil

Table 6 shows that bok choys grown in soil had phytol ($C_{20}H_{40}O$) as the highest AUC at 2,15%. All three samples of bok choys grown using different methods showed variation in their phytochemical content.

Bok Choy Plant Source	**IC_{50} (ppm)**	**Antioxidant Activity**
Plant Factory	147.15	Moderate
Hydroponic in a Greenhouse	1336,66	Weak
Soil Media	269,17	Weak

Table 7. Comparison of Bok Choy Antioxidant Activity

The DPPH test on antioxidant activity shown in Table 7 showed that bok choys grown in plant factory had the highest antioxidant activity, followed by bok choys grown in soil, and bok choys grown hydroponically in a greenhouse. Bok choys grown in factory had an IC_{50} value of 147,15ppm which according to the categorisation of antioxidant activity by [26] is considered moderate. Bok choys grown in soil had an IC_{50} value of 269,17ppm which is subsequently considered weak, while bok choys grown hydroponically in a greenhouse had an IC_{50} value of 1336,66ppm which is categorised as weak.

3.2 Discussion

Bok choys grown in three different agricultural methods showed a significant difference in plant height, stem diameter, and dry weight. This is attributed to a much greater supply of nutrients in the hydroponic solution used, hence bok choys grown in a plant factory and a greenhouse had an overall better growth than bok choys grown conventionally in the soil. A previous research done by Rizal [28]showed that bok choys grown using an AB-mix based hydroponic nutrient solution had the greatest height compared to bok choys grown using other medias. The AB-mix solution also has a profound impact on the number of leaves and stem diameter, which is in line with research done by Fau [32]whose results showed that bok choy plants grown hydroponically had a higher amount of leaves compared to bok choy plants grown in soil media. This difference in growth is attributed by nitrogen content of each media, where the hydroponic nutrient solution possesses a higher concentration of nitrogen compared to soil media. Nitrogen content in soil is categorised as Total-N which is a calculation of all soil within the soil, but not all of the nitrogen in soil can be absorbed by plants hence the amount of nitrogen actually absorbed by plants is much lower [27].

The amount of chlorophyll in the bok choy plants was affected by the lighting used. Bok choys in plant factory had the highest amount of chlorophyll (51,24) which used artificial lighting of RBW LED. According to Lin et al., [33]plants grown in a plant factory which used RBW LED lights had a higher chlorophyll content compared to plants which used other forms of lighting. Chlorophyll concentration is heavily tied to a plant's nitrogen and magnesium supply which in turn impact the fresh and dry weight of a plant. A higher emunt of chloroyphyll means that a plant may produce more photosyntate hence its fresh weight and dry weight will increase [28]. Despite higher temperatures in the greenhouse compared to other growth locations, bok choys grown in the greenhouse possessed the highest fresh and dry weight. According to Graamans et al., [34], plants grown hydroponically in a a greenhouse were bigger compared to plants grown in a plant factory due to higher temperatures which made an impact on transpiration and respiration. In higher temperatures, plant metabolism will increase in speed leading to an increased absorption of water and nutrients which can oftentimes lead to wilting. However, when a plant receives enough water and nutrients under these condition then it will result in an increase of biomass [29].

The GC-MS results of bok choys grown in the three different agricultural method all had neophytadiene as the phytochemical with the greatest peak. However, there were differences in the phytochemical content itself leading to differences in chemicals with the highest AUC. Bok choys grown in plant factory had the highest content of cyclopentylamine which possess antibacterial properties by inhibiting biofilm production which is needed to adhede onto plants [9]. Whereas in bok choys grown hydroponically in a greenhouse and in soil had a highest proportion of phytol, with bok choys grown in a greenhouse having a higher percentage. Phytol is a metabolite which has a role in chlorophyll synthesis and Vitamin E production, and it's production increased when plants undergo abiotic stress[30]. The difference in phytochemical content of the bok choys is mainly attributed to environmental factors where higher temperatures and higher light intentisty will often result in an increased production of secondary metabolites as a response [35], [36]. In terms of lighting, the colour of light also plays a role in stimulating secondary metabolite production where red and blue LED lights are known to increase phytochemical content and antioxidant activity in plants [31].

Bok choy plants grown in a plant factory had the greatest antioxidant activity and the highest amount of antioxidants with 9,96% of the total phytochemicals detected free-radical reducing properties, this was followed by bok choys grown in soil with phytochemicals with antioxidant properties encompassing 5,30% of the total chemicals, and finally bok choys grown hydroponically in a greenhouse which posssessed a percentage of phytochemicals with an antioxidant property at 3,74%. Aside from a

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higher percentage of antioxidants, bok choys grown in plant factory possessed strong antioxidants such as 3'-Methoxyacetophonone which is an antioxidant with anti-cancer effects and butylamine which can reduce oxidative stress in the brain [38], [39]. Meanwhile, bok choys grown in soil had a higher antioxidant content than bok choys grown hydroponically in a greenhouse with antioxidants such as palmitic acid and cyclononane which have a higher antioxidant activity compared to α-tocopherol [37], [23]. Antioxidant content of a plant increases when it is exposed to red and blue lights, which results in a bitter taste compared to plants with lower antioxidant content [31], hence one of the main factors in the bok choys grown in plant factory higher antioxidant content is lighting.

IV. CONCLUSIONS

Bok choys grown hydroponically in greenhouses had the greatest growth, followed by bokchoys grown in plant factory, and bokchoys grown conventionally in soil. There was a difference in the phytochemical contents of bokchoys grown using three different agricultural methods, with bokchoys grown in plant factory having the highest content of cyclononane, whilst bokchoys grown hydroponically in a greenhouse and conventionally in soil had phytol as their highest content. Bok choys grown in plant factory had the highest antioxidant content, categorized as having moderate antioxidant activity.

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