horticulturae

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Winter Production of Asian Leafy Greens in High Tunnels Using Biodegradable Mulches

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Abstract: Use of season extension tools such as high tunnels and diverse vegetable crops have been crucial in improving competitiveness of vegetable growers in Mississippi who operate on small- to medium-sized farms. Chinese cabbage, also known as pak choy or bok choy, has become increasingly popular due to numerous cultivar choices, fast maturity, high productivity, tolerance for frost, and its potential use for winter production in high tunnels in a subtropical climate. Five Chinese cabbage cultivars including 'Asian Delight', 'Black Summer', 'Red Pac', 'Rosie', and 'Tokyo Bekana' were evaluated for plant growth, yield, and mineral nutrient concentrations when grown with three types of biodegradable plastic mulches (BDMs) and one polyethylene (PE, or plastic) mulch in a high tunnel in two experiments from 30 October 2019 to 18 March 2020. The five tested cultivars varied in plant height, widths, leaf SPAD, fresh and dry plant weights, marketable yield, and macro- and micro-nutrient concentrations. 'Tokyo Bekana' produced the highest marketable yield and fresh and dry plant weights in both experiments. The three BDMs resulted in similar marketable yield and mineral nutrients in tested cultivars and similar temperatures of leaf, mulch, and substrate compared to the PE mulch. The high tunnel provides a viable way for the winter production of selected Chinese cabbage cultivars in a subtropical climate with possible different yields between production cycles due to varying microenvironment in those months.

Keywords: *Brassica rapa* var. chinensis; protected culture; yield; mineral nutrients; bioplastic mulch

Citation: Li, T.; Lalk, G.T.; Zhang, Q.; Xing, Z.; Bi, G. Winter Production of Asian Leafy Greens in High Tunnels Using Biodegradable Mulches. *Horticulturae* **2021**, *7*, 454. https://doi.org/10.3390/horticulturae7110454

Academic Editor: Daniel Drost

Received: 24 August 2021
Accepted: 30 October 2021
Published: 3 November 2021

1. Introduction

Vegetables are an important part of specialty crop production and agriculture in Mississippi [1,2]. Vegetable growers in the state generally operate on small- to medium-sized farms and market their produce locally through farmer's markets, community supported agriculture (CSA), local restaurants, and direct on-farm sale [3,4]. As growers seek to diversify their production and increase competitiveness at local markets, Asian leafy greens have become increasingly popular cool-season crops with their wide adaptability, high productivity, and relatively short maturity, along with many cultivar choices [5]. Asian leafy greens are suitable for fall or spring planting in the southern states of the United States (US). Non-heading Chinese cabbages (*Brassica rapa* var. chinensis), also known as pak choy or bok choy, are of the most grown Asian leafy greens. They perform the best with daily average temperatures of 13–21 °C, tolerate frost, and have the potential to be produced during the mild winter in southern US with some protection from season extension tools [5,6]. Selection of suitable cultivars for cold tolerance and productivity is crucial for winter production [7].

High tunnels, or hoop houses, are constructed with metal frames covered with polyethylene films, with no automatic cooling, heating, or lighting systems [8,9]. High tunnels raise air and soil temperatures, lower frost risks, and provide shield from rain, resulting in advanced harvests, increased yield, improved crop quality, and decreased disease pressure [4,10,11]. With elevated temperatures compared to outdoors, high tunnels can extend the growing season by 1 to 4 weeks in the spring and 2 to 8 weeks in the fall [7].

There has been a rapidly increasing interest among US growers in using high tunnels for early crop maturity and extended harvest seasons in the production of vegetable, cut flower, and small fruit crops [4,11–16]. Research-based recommendations for crop choices and cultural practices suitable to local climates in a high tunnel production system are in need.

Polyethylene mulches are globally used to cover raise beds for the benefits of increased soil temperature, weed control, moisture retention, and ultimately improved yield and quality of specialty crops [17,18]. However, extensive use of fuel-based PE films that are not biodegradable is not environmentally or economically sustainable, and can cause potential soil and water contaminations [19,20]. Removal and disposal of PE films are labor intensive and add significantly to production costs [21]. There have been increasing concerns of PE mulch not being completely removed, leaving remnants in soil for decades [22,23].

Biodegradable mulches have been investigated in recent years as a major sustainable alternative to PE films [24]. The global market for BDMs is expected to grow continuously with the increasing needs for eco-friendly substitutes to PE in horticulture [25]. They are made of different bio-based polymers derived from microbes or plants, including polylactic acid (PLA), starch, cellulose, and polyhydroxyalkanoates (PHA), or fossil-sourced materials including poly(butylene succinate) (PBS), poly(butylene succinate-co-adipate) (PBSA), and poly(butylene-adipate-co-terephthalate) (PBAT) [25–27]. Bioplastic mulches are designed to be tilled into soil after production and decompose in situ, or retrieved and composted. They should be completely catabolized by soil microorganisms, converted to microbial biomass, carbon dioxide (CO_2), and water [21–23,28–30], even though complete breakdown in a reasonable amount of time is not always reported [31]. The ultimate fate of BDMs in the soil and their long term effect on soil ecosystems remains unclear.

Biodegradable plastic mulches of various types were reported to result in similar crop yield and soil temperatures to PE film in the production of sweet corn (*Zea mays* L.), pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum*), zucchini (*Cucurbita pepo*), pumpkin (*Cucurbita pepo*), and muskmelon (*Cucumis melo*), etc. [32–35]. Most BDMs have lower tensile strength and mechanical resistance compared with PE films [36,37]. As a result, BDMs are expected to last one growing season, and are more subject to rips, tears, and holes during installation or in production [36,38]. Some BDMs are reported to crack not long after transplanting [39]. Conditions including high winds, temperature, greater solar radiation, and rainfall accelerate the deterioration of BDMs [38]. Due to early degradation, BDMs were shown to have reduced season-long weed control for persistent weed species such as nut sedge [21]. The use of BDMs for heavy fruit production such as pumpkin resulted in adhesion problem on the fruit and increased unmarketable yield [40]. The efficacy of using BDMs under high tunnels was not reported as much as in field production, which requires further investigation.

The objectives of this study were to: (1) Investigate feasibility of winter production of Asian leafy green cultivars in Mississippi with a high tunnel; (2) Investigate efficacy of three types of biodegradable plastic mulches compared to standard PE mulch in the production of Asian greens; and (3) Investigate yield and mineral nutrients of five Asian green cultivars affected by mulch type in the high tunnel.

2. Materials and Methods

2.1. Seedling Preparation

This study consists of two experiments from 30 October to 5 December 2019 and from 11 February to 18 March 2020. Five non-heading Chinese cabbage cultivars, 'Asian Delight', 'Black Summer', 'Red Pac', 'Rosie', and 'Tokyo Bekana,' were selected and purchased from Johnny's Selected Seeds (Fairfield, ME, USA). Seeds were planted into 96-cell trays in a greenhouse with temperature setting of 25 °C and natural light on campus of Mississippi State University (Starkville, MS, USA) on 18 September 2019 and 2 January 2020 for the first and second experiment, respectively. One week after germination, extra plants were cut off with a pair of scissors to leave one healthy transplant in each cell.

2.2. Plant Cultivation and Experimental Design

Seedlings (40–42 days old) were hardened off approximately one week prior to planting and transplanted into the high tunnel located at the R. R. Foil Plant Science Research Center of Mississippi State University (lat. 33.45° N, long. 88.79° W; USDA hardiness zone 8a) on 30 October2019 and 11 February 2020 for the first and second experiment, respectively. The high tunnel measured 29.0 m long and 9.1 m wide, oriented north to south, and was placed in full sun. The high tunnel had metal frames covered with 0.15 mm (6 mil) clear polyethylene film and had side curtains and two doors on end walls opening to 1.5 m and 3 m high, respectively (Tubular Structure, Lucedale, MS, USA). Within the experiment duration, side curtains and end doors of the high tunnel were closed when the air temperature was below 4.4 °C, and remained open otherwise with temperatures above 4.4 °C.

Due to the heavy clay soil in the high tunnel not being suitable for growing vegetables, five raised beds were constructed with composted pine bark that was introduced into the high tunnel, serving as five replications (blocks). Each raised bed measured 27.4 m long, 80 cm wide at the base, 45 cm wide at the top, 15 cm high, and was spaced 1.2 m center-to-center. Before mulch was laid onto the bed, granular lime at a rate of 2.96 kg·m^{-3} (Soil Doctor Pelletized Lawn Lime; Oldcastle, Atlanta, GA) and a controlled release fertilizer 15N-3.9P-10K (Osmocote® 15-9-12 plus, 3–4 months; ICL Specialty Fertilizers, Summerville, SC, USA) at a rate of 4.75 kg·m^{-3} were incorporated into the raised beds. One drip tape (15.9 mm in diameter, 0.91 L per hour; Netafim, Tel Aviv-Yafo, Israel) was laid onto the center of each raised bed with 30 cm emitter spacing.

Each raised bed was then divided into four equal sections approximately 6.9 m in length. Each section was randomly covered with one of the four mulch types. Three biodegradable plastic mulches (BDMs) and one black polyethylene (PE, also referred as plastic) film were tested in this study and are summarized in Table 1. Twelve plants from each cultivar were transplanted into the raised beds in staggered double rows 25 cm between plants and 25 cm between rows. This experiment was set up in a split-plot design with factorial arrangement of treatments and five replications. Mulch type served as the main plot factor and was randomly distributed within a block. Within each main plot, Asian green cultivar served as the sub-plot factor and was randomly distributed within each main plot. There were twelve single-plant subsamples in each sub plot.

Table 1. Product name, composition, and manufacturer information of the four types of mulch films used in this study.

Mulch Product	Mulch Composition and Characteristics [1,2]	Manufacturer
BioTelo Agri	Mater-Bi®; biodegradable and compostable; 15 µm; black	Dubois Agrinovation, Waterford, ON, Canada
Organix A.G.	BASF Ecovio (PBAT + PLA); biodegradable and compostable; 17.8 µm; black	Organix Solutions, Bloomington, MN, USA
WeedGuardPlus®	Corn starch and non-disclosed biopolymers; biodegradable and compostable; 15 µm; black	Sunshine Paper Co. LLC, Auroro, CO, USA
Plastic	Polyethylene; nondegradable; 32 µm; black	Filmtech Corp., Allentown, PA, USA

[1] Mulch composition information was gathered from manufacturer and references [21,37–39]. [2] PBAT = poly(butylene-adipate-co-terephthalate); PLA = polylactic acid.

All plants were drip irrigated daily as needed and were fertigated with 20N-8.7P-16.6K water-soluble fertilizer (Peters® Professional 20-20-20 General Purpose; ICL Specialty Fertilizers) at a rate of 100 ppm N through an injector (D14MZ2; Dosatron Intl. Inc., Clearwater, FL, USA) during the first two weeks after transplanting.

2.3. Microenvironment in the High Tunnel

Environmental conditions including air temperature, relative humidity (RH), and photosynthetically active radiation (*PAR*) were recorded in the high tunnel. A data logger (HOBO USB Micro Station H21-USB; Onset Computer Corp., Bourne, MA, USA) was installed in the center of the high tunnel. A temperature and RH sensor (HOBO S-THB-

M002; Onset Computer Corp.) and a quantum sensor (HOBO S-LIA-M003; Onset Computer Corp.) were connected to the data logger to monitor air temperature, RH, and *PAR* at one-hour intervals. Daily light integral (DLI) was calculated by multiplying average daily *PAR* with 0.0864 as described by Torres and Lopez [41]. Growing degree days were calculated daily by [(Daily maximum temperature + daily minimum temperature)/2–base temperature]. Cumulative GDDs between certain time periods were estimated by summing daily GDDs. The base temperature used for Asian greens was 4 °C [7]. The microenvironment in the high tunnel is presented in Figures S1–S3.

2.4. Data Collection and Plant Harvest

Before harvest, two plants from each sub-plot were randomly selected to measure plant height, widths, and relative leaf chlorophyll content, measured as Soil-Plant Analysis Development (SPAD) readings, on 4 December 2019 and 16 March 2020 in the first and second experiment, respectively. Plant width was calculated as the average of width 1 (widest points apart) and width 2 (width at perpendicular direction to width 1). Leaf SPAD was measured on three fully expanded leaves of each selected plant using a chlorophyll meter (SPAD 502 Plus; Konica Minolta, Inc., Osaka, Japan). Three readings measured from the selected leaves, one reading per leaf, were averaged to represent relative chlorophyll content of a given plant.

Temperatures on leaf surface, mulch surface, substrate temperature and moisture were also measured with two readings from each cultivar (or sub plot). Leaf surface temperature was measured on two plants in each plot, using a handheld infrared dual-laser thermometer (Southwire, Carrollton, CA, USA). Two mulch temperature readings were also collected in each sub-plot using the same thermometer. Substrate temperature at 10 cm depth was measured using a digital thermometer probe (Fisherbrand^{TM} Traceble^{TM}; Pittsburgh, PA, USA). Substrate moisture at 6 cm depth was measured using a soil moisture sensor (ML2x; Delta-T Devices, Cambridge, UK) toward the center of each plot with two readings from each sub-lot. The moisture sensor was connected to a soil sensor reader (HH2; Delta-T Devices) for instant moisture readings.

After plant growth and temperature data were collected, all plants were harvested at crown level to measure fresh yield on 5 December 2019 and 18 March 2020 for the first and second experiment, respectively. The twelve plants in each plot were separated into marketable yield and culled for unmarketable yield based on plant size and leaf quality. The number of plant in each category was also recorded. Besides total yield, two marketable plants were measured for individual fresh plant weight, and oven dried at 60 °C until constant weight. The dry weight of each plant was then measured.

2.5. Mineral Nutrient Analyses

Each dry plant was ground to pass through a 1 mm sieve with a grinder (Wiley mini mill, Thomas Scientific, Swedesboro, NJ, USA) for mineral nutrient analyses. Combustion analysis was used for the determination of total nitrogen (N) concentration with 0.25 g of dry tissue using an elemental analyzer (vario MAX cube; Elementar Americas Inc., Long Island, NY, USA). A dry tissue sample of 0.5 g was digested with 1 mL of 6 M hydrochloric acid (HCl) and 50 mL of 0.05 M HCl to obtain the concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and boron (B) using inductively coupled plasma optical emission spectrometry (SPECTROBLUE; SPECTRO Analytical Instruments, Kleve, Germany). Plant samples were tested at the Mississippi State University Extension Service Soil Testing Laboratory. Concentrations of macronutrients ($mg \cdot g^{-1}$) and micronutrients ($\mu g \cdot g^{-1}$) in Asian green plants are presented on a dry weight basis.

2.6. Statistical Analyses

Two-way analysis of variance (ANOVA) was performed on all tested variables. All data were analyzed using the PROC GLMMIX procedure of SAS (version 9.4; SAS Institute, Cary, NC, USA). Where indicated by ANOVA, means were separated using Tukey's Honest Significant Difference (HSD) test at $p < 0.05$. Data from the two experiments were compared as repeated measures, where the experiment date was used as a factor to analyze its effect.

3. Results

The experiment date affected all measured dependent variables in this study, showing varying trends between the November and February experiments (data not shown). Therefore, data from the two experiments are presented separately.

3.1. Plant Height, Widths, and Leaf SPAD

The Asian green cultivars varied in plant height, width, and relative leaf chlorophyll content, measured as SPAD readings in both experiments. Mulch type did not affect any of the three variables in either experiment (Table 2).

Table 2. Plant height, width, and leaf SPAD in five Asian green cultivars grown with four types of mulches in a high tunnel in Starkville, MS in two experiments.

	November 2019 [1,2]			February 2020		
Cultivar	**Plant Height**	**Plant Width**	**SPAD**	**Plant Height**	**Plant Width**	**SPAD**
	(cm)	**(cm)**		**(cm)**	**(cm)**	
Asian Delight	9.65 c	31.0 b	50.3 c	11.2 e	27.1 d	52.1 b
Black Summer	16.3 b	38.8 a	63.1 a	19.1 c	34.1 b	60.8 a
Red Pac	16.1 b	33.6 b	57.0 b	17.9 d	32.0 c	53.2 b
Rosie	17.1 b	33.1 b	47.1 d	20.5 b	35.0 b	46.3 c
Tokyo Bekana	21.9 a	33.2 b	22.7 e	33.6 a	44.8 a	21.3 d
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Mulch	0.16	0.72	0.80	0.68	0.96	0.84
Cultivar * Mulch [3]	0.18	0.99	0.54	0.69	0.55	0.40

[1] Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at $p \leq 0.05$. [2] Means for a certain cultivar were obtained by averaging data from all four mulch types. [3] * suggests interaction between cultivar and mulch.

Among the five tested cultivars, 'Tokyo Bekana' plants had the largest sizes in terms of plant height and widths in both experiments. 'Asian delight' was the most dwarfed cultivar with the least heights of 9.65 cm and 11.2 cm in the November 2019 and February 2020 experiments, respectively. The cultivars 'Black Summer', 'Red Pac', and 'Rosie' had intermediate heights of 16.1 cm to 17.1 cm in November and 17.9 cm to 20.5 cm in February, respectively. In the November experiment, 'Black Summer' produced the highest width of 38.8 cm, higher than other four cultivars with similar widths of 31.0 cm to 33.6 cm. In the February experiment, 'Tokyo Bekana' had the highest width of 44.8 cm and 'Asian Delight' had the lowest width of 27.1 cm.

For leaf SPAD measurement, 'Black Summer' had the highest leaf SPAD of 63.1 and 60.8, with 'Tokyo Bekana' having the lowest leaf SPAD readings of 22.7 and 21.3 in the November and February experiment, respectively. The three cultivars 'Asian Delight', 'Red Pac', and 'Rosie' had intermediate leaf SPAD of 47.1 to 57.0 in November and 46.3 to 53.2 in the February experiment, respectively.

3.2. Fresh and Dry Plant Weights

Fresh and dry plant weights varied among cultivars in both experiments (Table 3). Mulch type affected fresh and dry plant weights in November 2019 (Table 4).

Table 3. Fresh, dry plant weights, and yields of five Asian green cultivars grown in a high tunnel with four types of mulches in Starkville, MS in two experiments.

Cultivar	November 2019 [1,2]				February 2020			
	Fresh Weight	Dry Weight	Marketable YIELD	Unmarketable Yield	Fresh Weight	Dry Weight	Marketable Yield	Unmarketable Yield
	($g \cdot plant^{-1}$)	($g \cdot plant^{-1}$)	($kg \cdot ha^{-1}$)	($kg \cdot ha^{-1}$)	($g \cdot plant^{-1}$)	($g \cdot plant^{-1}$)	($kg \cdot ha^{-1}$)	($kg \cdot ha^{-1}$)
Asian Delight	127.6 c	6.91 c	3596 b	157.6 b	262.1 c	7.99 bc	10,837 b	18.4
Black Summer	155.3 b	8.34 b	3834 b	769.9 a	305.2 b	10.3 b	11,959 b	159.3
Red Pac	74.0 d	4.75 d	1434 c	137.0 b	145.7 d	7.82 bc	4500 c	116.8
Rosie	79.7 d	4.78 d	1883 c	76.6 b	139.2 d	6.64 c	5442 c	66.5
Tokyo Bekana	237.1 a	10.98 a	5510 a	81.3 b	445.9 a	13.9 a	14,915 a	73.4
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.324
Mulch	0.031	0.0042	0.094	0.095	0.49	0.096	0.75	0.40
Cultivar * Mulch [3]	0.13	0.052	0.93	0.051	0.57	0.67	0.93	0.46

[1] Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at $p \leq 0.05$.
[2] Means for a certain cultivar were obtained by averaging data from all four mulch types. [3] * suggests interaction between cultivar and mulch.

Table 4. Fresh and dry plant weights, substrate temperature, moisture, and leaf temperature affected by mulch type in the production of Asian leafy greens in a high tunnel in the November and February experiment.

Mulch	November 2019 [1,2]				February 2020
	Fresh Weight	Dry Weight	Substrate Temp.	Substrate Moisture	Leaf Temp.
	($g \cdot plant^{-1}$)	($g \cdot plant^{-1}$)	(°C)	(%)	(°C)
BioTelo Agri	148.4 a	8.07 a	14.7 a	16.8 b	17.7 a
Organix A.G.	121.4 b	6.40 b	14.6 a	17.5 ab	17.8 a
WeedGuardPlus®	130.7 ab	7.01 ab	14.7 a	17.6 ab	18.6 a
Plastic	138.5 ab	7.12 ab	14.9 a	19.0 a	18.8 a
p-value	0.031	0.0042	0.033	0.035	0.020

[1] Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at $p \leq 0.05$.
[2] Means for a certain mulch type were obtained by averaging data from all five tested cultivars.

'Tokyo Bekana' plants had the largest fresh and dry weights of 237.1 $g \cdot plant^{-1}$ and 10.98 $g \cdot plant^{-1}$ in November and 445.9 $g \cdot plant^{-1}$ and 13.9 $g \cdot plant^{-1}$ in February, respectively, higher than the other four cultivars. Ranking of fresh plant weight was similar among cultivars in both experiments: 'Tokyo Bekana' > 'Black Summer' > 'Asian Delight' > 'Red Pac', or 'Rosie'. Dry weights of 'Red Pac' and 'Rosie' were the lowest, at 4.75 $g \cdot plant^{-1}$ and 4.78 $g \cdot plant^{-1}$ among cultivars in November. Dry weights of 'Asian Delight', 'Red Pac', and 'Rosie' were similar, ranging from 6.64 $g \cdot plant^{-1}$ to 7.99 $g \cdot plant^{-1}$ in the February experiment.

Mulch type generally resulted in similar fresh or dry plant weights, except that BioTelo increased fresh and dry plant weights of 148.4 $g \cdot plant^{-1}$ and 8.07 $g \cdot plant^{-1}$ weight when compared to Organix A.G. with 121.4 $g \cdot plant^{-1}$ and 6.4 $g \cdot plant^{-1}$ fresh and dry weights in the November experiment (Table 4).

3.3. Marketable and Unmarketable Yields

Marketable yield in both experiments and unmarketable yield in November 2019 varied among cultivars. Mulch type did not affect marketable or unmarketable yield in either experiment (Table 3).

Trends of marketable yield among cultivars were similar in both experiments, where 'Tokyo Bekana' produced the highest marketable yields of 5510 $kg \cdot ha^{-1}$ in November and 14,915 $kg \cdot ha^{-1}$ in February, higher than any other cultivars. Lower, 'Asian Delight' and 'Black Summer' produced similar intermediate yields of 3596 $kg \cdot ha^{-1}$ and 3834 $kg \cdot ha^{-1}$ in November, and 10,837 $kg \cdot ha^{-1}$ and 11,959 $kg \cdot ha^{-1}$ in February, respectively. The two red-leaf cultivars 'Red Pac' and 'Rosie' produced the lowest yields of 1434 $kg \cdot ha^{-1}$ and 1883 $kg \cdot ha^{-1}$ in November, and of 4500 $kg \cdot ha^{-1}$ and 5442 $kg \cdot ha^{-1}$ in February, respectively.

Unmarketable yield ranged from 76.6 kg·ha^{-1} to 769.9 kg·ha^{-1} in November, equivalent to 1.45% to 16.7% of total yield. 'Black summer' produced the highest unmarketable yields in November, with the main reason observed to be malformed leaves. There was no difference in unmarketable yield among cultivars in the February experiment due to large variations, ranging from 18.4 kg·ha^{-1} to 159.3 kg·ha^{-1} equivalent of 0.17% to 2.53% of total yield.

3.4. Temperatures of Mulch, Leaf, Substrate, and Substrate Moisture

Mulch temperature varied among cultivars but was not affected by mulch type, ranging from 22.5 °C in 'Red Pac' to 25.6 °C in 'Tokyo Bekana' in the November experiment and from 14.7 °C in 'Tokyo Bekana' to 20.8 °C in 'Asian Delight' plot in the February experiment (Table 5).

Table 5. Temperatures of mulch, leaf, substrate, and substrate moisture in five Asian green cultivars grown with four types of mulches in a high tunnel in Starkville, MS in two experiments.

	November 2019 [1,2]				February 2020			
Cultivar	**Mulch Temp.**	**Leaf Temp.**	**Substrate Temp.**	**Substrate Moisture**	**Mulch Temp.**	**Leaf Temp.**	**Substrate Temp.**	**Substrate Moisture**
	(°C)	**(°C)**	**(°C)**	**(%)**	**(°C)**	**(°C)**	**(°C)**	**(%)**
Asian Delight	23.5 ab	11.5 ab	14.7 a	18.1 a	20.8 a	18.0 bc	18.8 a	21.4 a
Black Summer	24.4 ab	11.2 ab	14.6 ab	18.0 a	17.3 b	17.7 c	18.3 b	22.4 a
Red Pac	22.5 b	12.7 a	14.9 a	18.3 a	19.2 ab	20.0 a	19.1 a	20.8 a
Rosie	23.2 ab	11.6 ab	14.7 a	17.8 a	18.7 b	19.0 ab	19.0 a	21.2 a
Tokyo Bekana	25.6 a	10.6 b	14.3 b	16.5 a	14.7 c	16.4 d	17.7 c	22.9 a
Cultivar	0.027	0.0081	0.0005	0.265	<0.0001	<0.0001	<0.0001	0.16
Mulch	0.66	0.055	0.033	0.035	0.098	0.020	0.17	0.065
Cultivar * Mulch [3]	0.80	0.079	0.33	0.21	0.75	0.54	0.35	0.092

[1] Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at $p \leq 0.05$. [2] Means for a certain cultivar were obtained by averaging data from all four mulch types. [3] * suggests interaction between cultivar and mulch.

Leaf temperatures varied among cultivars in both experiments, showing no difference among mulch types even with a P-value of 0.02 in the February experiment (Tables 4 and 5). Leaf temperature was generally similar among cultivars, ranging from 10.6 °C in 'Tokyo Bekana' to 12.7 °C in 'Red Pac' in November. In the February experiment, 'Red Pac' and 'Rosie' had comparable highest leaf temperatures of 20.0 °C and 19.0 °C, higher than 'Black Summer' or 'Tokyo Bekana' with leaf temperatures of 17.7 °C and 16.4 °C, respectively.

Substrate temperature at 10 cm-depth ranged from 14.3 °C to 14.7 °C in the November experiment, generally similar among cultivars (Table 5). In the February experiment, the highest substrate temperatures of 18.8 °C to 19.1 °C were found in cultivars 'Asian Delight', 'Red Pac', and 'Rosie', with the lowest substrate temperature of 17.7 °C in 'Tokyo Bekana'. An intermediate substrate temperature of 18. 3 °C was found in 'Black Summer' plot. Even with a p-value of 0.033 in the November experiment, multiple comparison showed no difference in substrate temperature among mulch types (Table 4).

Substrate moisture at 6 cm depth ranged from 16.5% to 18.3% in November, and 20.8% to 22. 9% in February, with no difference among cultivars (Table 5). Plastic mulch resulted in higher substrate moisture of 19.0% than BioTelo of 16.8%, but similar moisture level to Organix A.G. or WeedGuardPlus (Table 4).

3.5. Macronutrients

Concentrations of macronutrients including N, P, K, Ca, and S in the November experiment and N, P, K, Mg, and S in the February experiment varied among cultivars (Table 6). The five tested cultivars had similar Mg concentrations in November and similar

Ca concentrations in February. Mulch type did not affect macronutrient concentrations in tested Asian green cultivars.

Table 6. Macronutrient concentrations in five Asian green cultivars grown with four types of mulches in a high tunnel in Starkville, MS in two experiments.

	November 2019 [1,2]						February 2020					
Cultivar	**N [3]**	**P**	**K**	**Ca**	**Mg**	**S**	**N**	**P**	**K**	**Ca**	**Mg**	**S**
			(mg·g^{-1})						(mg·g^{-1})			
Asian Delight	50.3 a	7.24 a	47.5 b	17.6 ab	3.64 a	5.84 b	59.0 a	8.27 a	52.2 b	24.3 a	4.84 a	5.97 b
Black Summer	46.3 b	7.11 a	49.0 ab	15.6 b	3.35 a	7.18 a	55.1 ab	7.94 a	58.7 ab	19.6 a	4.38 ab	7.30 a
Red Pac	50.4 a	5.82 b	47.6 b	17.0 ab	3.41 a	5.79 b	58.8 a	6.50 bc	62.6 ab	21.0 a	4.58 ab	6.83 ab
Rosie	49.5 a	5.72 b	48.0 b	17.8 ab	3.57 a	5.67 b	59.5 a	5.91 c	58.2 ab	20.7 a	4.04 b	6.35 ab
Tokyo Bekana	41.3 c	7.91 a	55.4 a	20.4 a	3.71 a	6.18 b	49.4 b	7.81 ab	67.3 a	21.3 a	4.15 ab	6.51 ab
Cultivar	<0.0001	<0.0001	0.0034	0.0099	0.27	<0.0001	<0.0001	<0.0001	0.0046	0.10	0.025	0.042
Mulch	0.72	0.75	0.15	0.57	0.34	0.50	0.34	0.45	0.44	0.99	0.89	0.40
Cultivar * Mulch [4]	0.59	0.94	0.91	0.99	0.99	0.72	0.054	0.066	0.70	0.061	0.49	0.42

[1] Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at $p \leq 0.05$.
[2] Means for a certain cultivar were obtained by averaging data from all four mulch types. [3] N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur. [4] * suggests interaction between cultivar and mulch.

'Asian Delight', 'Red Pac', and 'Rosie' had similar highest N concentrations of 49.5 mg·g^{-1} to 50.4 mg·g^{-1} in November and of 58.8 mg·g^{-1} to 59.5 mg·g^{-1} in February, respectively. 'Tokyo Bekana' had the lowest N concentrations among cultivars in both experiment, 41.3 mg·g^{-1} in November and 49.4 mg·g^{-1} in February. Nitrogen concentration in 'Black Summer' was 46.3 mg·g^{-1} in November and 55.1 mg·g^{-1} in February, respectively.

'Asian Delight', 'Black Summer', and 'Tokyo Bekana' had similar P concentrations of 7.11 mg·g^{-1} to 7.91 mg·g^{-1} in November and 7.81 mg·g^{-1} to 8.27 mg·g^{-1} in February, higher than 'Rosie' with P concentrations of 5.72 mg·g^{-1} and 5.91 mg·g^{-1} in November and February, respectively. Phosphorus concentration in 'Red Pac' was 5.82 mg·g^{-1} in November and 5.91 mg·g^{-1} in February, respectively.

Potassium concentrations ranged from 47.5 mg·g^{-1} in 'Asian Delight' to 55.4 mg·g^{-1} in 'Tokyo Bekana' in November, and from 52.2 mg·g^{-1} in 'Asian Delight' and 67.3 mg·g^{-1} in 'Tokyo Bekana' in February, with four out of five cultivars having similar K concentrations.

Calcium concentration was generally similar among cultivars ranging from 15.6 mg·g^{-1} in 'Black Summer' to 20.4 mg·g^{-1} in 'Tokyo Bekana' in November, except that 'Tokyo Bekana' had higher Ca concentration than 'Black Summer'. There was no difference in Ca concentration among cultivars, or mulch type in the February experiment.

Magnesium concentration was similar among cultivars ranging from 3.35 mg·g^{-1} to 3.64 mg·g^{-1} in November. In February, Mg concentration ranged from 4.04 mg·g^{-1} in 'Rosie' to 4.84 mg·g^{-1} in 'Asian Delight', with cultivars generally having similar Mg concentrations except that 'Asian Delight' had higher Mg concentration than 'Rosie'.

In November, 'Black Summer' had higher S concentration of 7.18 mg·g^{-1} than the other four cultivars with similar S concentrations of 5.67 mg·g^{-1} to 6.18 mg·g^{-1}. In February, S concentration ranged from 5.97 mg·g^{-1} in 'Asian Delight' to 7.3 mg·g^{-1} in 'Black Summer', with cultivars generally having similar S concentrations except for 'Black Summer' having a higher S concentration than 'Asian Delight'.

3.6. Micronutrients

Concentrations of micronutrients including Cu, Fe, Mn, Zn, and B in both experiments varied among cultivars. Mulch type did not affect micronutrient concentrations in tested Asian green cultivars (Table 7).

Table 7. Micronutrient concentrations in five Asian green cultivars grown with four types of mulches in a high tunnel in Starkville, MS in two experiments.

	November 2019 [1,2]					February 2020				
Cultivar	**Cu [3]**	**Fe**	**Mn**	**Zn**	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**B**
			(μg·g^{-1})					**(μg·g^{-1})**		
Asian Delight	2.57 a	85.2 a	90.7 b	59.0 a	33.1 ab	1.38 a	95.0 a	98.0 ab	60.3 a	34.4 b
Black Summer	1.22 b	72.4 b	78.7 b	56.7 a	29.0 b	1.18 ab	70.2 b	74.3 c	49.5 b	31.0 c
Red Pac	1.05 b	74.9 b	70.7 b	47.2 b	30.7 b	0.98 b	62.8 b	78.0 bc	42.9 c	34.4 b
Rosie	1.05 b	68.7 b	79.9 b	49.1 b	36.1 a	1.08 ab	60.6 b	89.1 abc	50.7 b	38.5 a
Tokyo Bekana	1.52 b	72.2 b	123.6 a	53.6 ab	36.5 a	0.98 b	66.5 b	107.1 a	52.5 b	39.8 a
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.018	<0.0001	0.0004	<0.0001	<0.0001
Mulch	0.063	0.83	0.031	0.57	0.86	0.46	0.054	0.19	0.66	0.28
Cultivar * Mulch [4]	0.63	0.92	0.79	0.79	0.98	0.32	0.095	0.062	0.053	0.071

[1] Different lower case letters suggest significant difference among means within a column indicated by the Tukey's HSD test at $p \leq 0.05$.
[2] Means for a certain cultivar were obtained by averaging data from all four mulch types. [3] Cu = copper; Fe = iron; Mn = manganese; Zn = zinc; B = boron. [4] * suggests interaction between cultivar and mulch.

'Asian Delight' had a higher Cu concentration of 2.57 μg·g^{-1} in November than the other four cultivars with similar Cu concentrations ranging from 1.05 μg·g^{-1} to 1.52 μg·g^{-1}. In February, 'Asian Delight', 'Black Summer', and 'Rosie' had similar Cu concentrations of 1.08 μg·g^{-1} to 1.38 μg·g^{-1}, while 'Red Pac' and 'Tokyo Bekana' had equal Cu concentration of 0.98 μg·g^{-1}.

Trend for Fe concentration was similar in both experiments, where 'Asian Delight' had higher Fe concentrations of 85.2 μg·g^{-1} in November and 95.0 μg·g^{-1} in February than the other four cultivars with similar Fe concentrations of 68.7 μg·g^{-1} to 74.9 μg·g^{-1} and 60.6 μg·g^{-1} to 70. 2 μg·g^{-1} in November and February, respectively.

Manganese concentration was the highest of 123.6 μg·g^{-1} in 'Tokyo Bekana', higher than other cultivars with similar Mn concentrations of 70.7 μg·g^{-1} to 90.7 μg·g^{-1} in November. In the February experiment, 'Asian Delight', 'Rosie', and 'Tokyo Bekana' had comparable highest Mn concentrations of 89.1 μg·g^{-1} to 107.1 μg·g^{-1}. 'Tokyo Bekana' had a higher Mn concentration than 'Black Summer' at 74.3 μg·g^{-1} or 'Red Pac' at 78.0 μg·g^{-1} in February.

Zinc concentration was the highest in 'Asian Delight' and 'Black Summer' at 59.0 μg·g^{-1} and 56.7 μg·g^{-1} in November, higher than 'Red Pac' or 'Rosie' with Zn concentrations of 47.2 μg·g^{-1} and 49.1 μg·g^{-1}, respectively. In the February experiment, 'Asian Delight' also had the highest Zn concentration of 60.3 μg·g^{-1}, higher than 'Black Summer', 'Rosie', or 'Tokyo Bekana' with similar Zn concentrations of 49.5 μg·g^{-1} to 52.5 μg·g^{-1}. 'Red Pac' had the lowest Zn concentration of 42.9 μg·g^{-1} in the February experiment.

For B concentration, 'Rosie' and 'Tokyo Bekana' had comparable highest B concentrations of 36.1 μg·g^{-1} and 36.5 μg·g^{-1}, higher than 'Black Summer' or 'Red Pac' with similar B concentrations of 29.0 μg·g^{-1} and 30.7 μg·g^{-1} in November. In the February experiment, 'Rosie' and 'Tokyo Bekana' also had comparable highest B concentrations of 38.5 μg·g^{-1} and 39.5 μg·g^{-1}, with 'Black Summer' having the lowest B concentration of 31.0 μg·g^{-1}. 'Asian Delight' and 'Red Pac' had equal intermediate B concentrations of 34.4 μg·g^{-1} in February.

4. Discussion

The microenvironment varied between the two experiments in terms of air temperatures, RH, and DLI (Figures S1–S3). Repeated measure showed higher soil temperature in raised beds in February than November in each cultivar plot (data not shown). Using 4 °C as the base temperature, the cumulative growing degree days (GDDs) in the November experiment was 245 GDDs, compared to 352 GDDs in the February experiment. The daily light integral ranged from 2.71 mol·m^{-2}·d^{-1} on 14 November to 25.1 mol·m^{-2}·d^{-1} on 1 November 2019 in the first experiment, and from 3.66 mol·m^{-2}·d^{-1} on 4 March to

30.9 mol·m^{-2}·d^{-1} on 6 March 2020 in second experiment. There were nine and five days with minimum air temperature below 0 °C in the November and February experiment, with the lowest minimum temperature of −3.21 °C and −3.57 °C, respectively (Figure S1). The Chinese cabbage cultivars are known to tolerate frost [6]. We did not observe visual damage of plants in either experiment. The high tunnel provides a viable way for winter production of Asian greens in Southeastern US.

As a result of varying microenvironment between the two experiments, the marketable yield of each cultivar in the February experiment was significantly higher than that in the November experiment (data not shown), averaging 2.71 (in 'Tokyo Bekana') to 3.14 (in 'Red Pac') times the marketable yield of those in the November experiment. Fresh plant weight was also significantly higher in the February experiment (data not shown), averaging 1.66 to 1.97 times of those in November. Such differences in yield were found when all Asian green cultivars were harvested at 36 days after planting (DAP) in both experiments. Fresh plant weights of tested Asian green cultivars were higher or similar to reported ranges when leafy green cultivars were produced in Northwest US during winter months, with a much longer growth period, harvested at 71 DAP [7]. Growers could expect different yields between production cycles during cool seasons and could possibly increase the growth period to increase yields during cooler months. On the other hand, growing Asian green cultivars in warmer months can result in faster harvest for satisfactory yield.

The growing density in the current study was 18,182 plants per acre (equivalent to 44,944 plants per hectare) in the high tunnel. This is in general agreement with the reported planting density of 14,520 plants per acre with leafy greens including mustard (*Brassica juncea*), kale (*Brassica oleracea* acephala group), and collard (*Brassica oleracea* acephala group) varieties with a slightly higher spacing of 38 cm [42], compared to the 25 cm spacing used in the current study. With similar density among cultivars, yield was mainly a function of fresh plant weight. Yields of red leaf cultivars 'Red Pac' and 'Rosie' can potentially be increased by increasing planting density due to their vertical growth habits and small crown widths.

Leafy green vegetables including bok choy cultivars are known to be a rich source of mineral nutrients. Macro- and micro-nutrient concentrations in tested bok choy cultivars were generally in agreement with reported ranges [43]. Besides basic nutrition, bok choys contain high levels of fiber and abundant bioactive compounds including many B-vitamins and carotenoids-antioxidants that play roles in blocking early stages of cancer [44]. Concentrations of phytochemicals affected by cultural practices, including the high tunnel and biodegradable mulches, merits further investigation.

The three tested BDMs resulted in similar fresh and dry plant weights and marketable yields compared to the plastic mulch. However, BioTelo increased fresh and dry plant weights compared to Organix A.G. in the November experiment. Concentrations of all tested macro- and micro-nutrients varied among cultivars, but were not affected by mulch type. Therefore, plastic much and BDMs resulted in similar fresh yield and quality of tested Asian green cultivars in a high tunnel in the current study. This is in agreement with multiple reports in the production of cucumber (*Cucumis sativus*), sweet corn, tomato, strawberry (*Fragaria* × *ananassa*), and pepper in open field or high tunnel systems [21,37–39,45].

In terms of the microenvironment, plastic mulch resulted in higher substrate moisture, of 19.0%, than BioTelo, of 16.8%, but similar substrate moisture to Organix A.G. or WeedGuardPlus, of 17.5% and 17.6%, respectively, in the November experiment. This could result from the higher permeability and low thickness of BDMs, as discussed by Ghimire et al. [39]. Even with *P*-values lower than 0.05 in substrate temperature measured on December 3 2019 and leaf temperature measured on March 16 2020, multiple comparison showed no difference among mulch types. Similar soil temperatures were also found in raised beds covered with BDMs compared to standard plastic mulch [35]. When using BDMs in the production of leafy greens, irrigation program may need to be adjusted to compensate for possible increased moisture loss through biodegradable mulches.

Strengths of BDMs are reported to be lower than PE mulch [46]. Degradation of BDMs was reported to provide poor weed control for persistent weed species in pepper production [21]. We did not observe any tearing problems during laying or in production, or weed growth penetrating the BDMs, even when they were thinner (15 to 17.8 μm) than the plastic film (32 μm). The Organix A.G. mulch was reported to split shortly after layering and resulted in poor weed control when used to produce sweet corn in a Mediterranean-type climate with average air temperatures of 16.1 °C [39]. The different results are likely due to the short production cycle of leafy greens (36 days) in the current study and that the high tunnel reduces wind, rainfall, and solar radiation, all of which accelerate deterioration of BDMs. This agrees with Miles et al. [38] reporting higher values of rips, tears, and percent of visually observed deterioration in open fields than high tunnels.

One advantage of using biodegradable mulch to compensate for the high initial purchase cost is saving of labor and mechanics in mulch removal, because they are designed to be tilled into the soil or composted at the end of the growing season [47]. Some BDMs were reported to deteriorate by 65% to 100% 397 days after soil incorporation, whereas other types showed little deterioration over the same time [33]. However, high deterioration of BDMs measured as percent soil exposure does not necessarily suggest degradation. Soil burial tests showed a high percentage of the BDM area remained after 18 month [46]. A number of factors, biotic or abiotic, including water, oxygen, temperature, mulch thickness, and microbial community size and composition, impact the breakdown of BDMs [26,30,48,49].

The objectives in our study were to examine crop yield and quality of Asian green cultivars using BDMs versus PE mulch, without investigating deterioration after soil incorporation. Use of a high tunnel for the production of vegetable crops, especially for small growers in the state of MS, relies on fast turnaround of high tunnel growing space and maximum occupancy of various crops throughout the year. It remains unclear whether the timeframe of mulch biodegradation will interfere with planting of another crop shortly after one is harvested, considering that deterioration and degradation of biodegradable mulches are likely slower in a high tunnel production system compared to an outdoor environment, as described above. Best management practices in a high tunnel production system using BDMs require further investigation.

5. Conclusions

The five tested Chinese cabbage cultivars varied in plant height, widths, leaf SPAD, fresh and dry plant weights, marketable yields, and macro- and micro-nutrient concentrations, with 'Tokyo Bekana' producing the highest marketable yield and fresh and dry plant weights in both experiments. The three BDMs resulted in similar marketable yields and mineral nutrients in tested cultivars and similar microenvironment including mulch, substrate, and leaf temperatures compared to the plastic mulch without tearing problems in the short production cycle of Asian greens. The high tunnel provides a viable way for winter production of selected Chinese cabbage cultivars in a subtropical climate with marketable yield being significantly higher during warmer months with higher GDDs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7110454/s1, Figure S1: Daily maximum, average, and minimum air temperatures in the high tunnel within the two experiment durations from 30 October to 4 December 2019 (A) and from 11 February to 18 March 2020 (B) in Starkville, Mississippi, Figure S2: Daily average relative humidity in the high tunnel within the two experiment durations from 30 October to 4 December 2019 (A) and from 11 February to 18 March 2020 (B) in Starkville, Mississippi, Figure S3: Daily light integral in the high tunnel within the two experiment durations from 30 October to 4 December 2019 (A) and from 11 February to 18 March 2020 (B) in Starkville, Mississippi.

Author Contributions: Conceptualization, T.L.; investigation, G.T.L., Q.Z., Z.X. and T.L.; writing—original draft preparation, T.L.; writing—review and editing, T.L. and G.B.; funding acquisition, T.L. and G.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported in part by the United States Department of Agriculture (USDA) Mississippi Department of Agriculture and Commerce Specialty Crop Block Grant Program (G00003962) and the United States Department of Agriculture (USDA) National Institute of Food and Agriculture Hatch Project MIS-112040. Mention of a trademark, proprietary product, or vendor, does not constitute a guarantee or warranty of the product by Mississippi State University or the USDA and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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