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Research Article

Productivity and Elemental/Chlorophyll Composition of Collard Greens in an Aquaponic System at Different Combinations of Media and Black Soldier Fly (*Hermetia illucens*) Larvae Frass Supplementations

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Black soldier fly (*Hermetia illucens*) larvae (BSFL) production will likely increase to meet the growing demand for sustainable protein and lipids. A by-product is "frass," which is a mixture of mostly larval excrement but also chitin. This mineral-richby-product could be a valuable aquaponic supplement, but the information is limited. The aim of this study was twofold to determine if BSFL frass additions could enhance the production, mineral composition, and chlorophyll content of collard greens (*Brassica oleracea* var. *viridis*) after 10 weeks as well as the influence of culturing collard greens with or without media. Each aquaponic system contained 20 channel catfish (*Ictalurus punctatus*; initial mean weight of 128 g) that were fed twice daily to apparent satiation. Results showed that dissolved nitrogen, pH, and dissolved oxygen were unaffected ($p > 0.05$) by BSFL frass additions. There was a significant media and BSFL frass effect ($p < 0.05$) on total weight, individual weight, and total height of collard greens where the media/frass treatment led to a ninefold increase in weight compared to the no media/no frass treatment. Moreover, both media and BSFL frass significantly increased ($p < 0.05$) the total chlorophyll content while extensive chlorosis was observed in the no media/no frass group. Manganese in collard greens was significantly increased ($p < 0.05$) by BSFL frass. The best combination for collard green growth and preventing any chlorosis was the media/frass treatment, which may be due to an increase in various nutrients that were more accessible by biomineralization in the media.

1. Introduction

The combination of farming fish with plants is known as aquaponics, and this integration is designed to be mutually symbiotic to both the fish and plants [1, 2]. In traditional recirculating systems, dissolved fish waste can eventually accumulate to toxic levels and thus requireswater exchange. In an aquaponic system, however, the dissolved waste is taken up as essential nutrients by the plants leading to two sellable products (fish and the plants) [1, 3]. Nitrogen, mostly in the form of nitrate, is often in abundance for aquaponic growth, but sometimes, there are insufficient essential macro- and micro-nutrients. Therefore, it is common for aquaponic farmers to supplement with additional nutrients, and the most common include iron (Fe, in chelated form) as well as potassium (K) and calcium (Ca) that are often in the form of the buffers $CaCO_3$ and K_2CO_3, respectively [4]. While Fe, K, and Ca are often limiting nutrients in aquaponic systems [5], this may depend on the system type and composition of aquafeeds as well as fish and plant species. For example, Yang and Kim [4] found that magnesium (Mg) and Ca were limiting nutrients during the aquaponic culture of tomatoes and herbs, and these deficiencies were linked to "chlorosis." Chlorosis is quantified by the amount of chlorophyll and is easily observed visually based on the yellowing of the leaves, which reduces nutrient uptake and growth [6, 7]. The amount of the most limiting nutrients is proportional to productivity, and therefore, identifying the limiting nutrient(s) is important to optimizing productivity. Various aquaponic supplements are commercially available that widely range in price, composition, and type (e.g., organic versus inorganic), but ideally, these should be cost-effective, readily available, contain accessible nutrients by the plants, as well as being safe to both plants and fish.

Insect "frass," or manure, is receiving increased attention as an organic fertilizer due to this being rich in various essential plant nutrients as well as containing chitin that reportedly may have prebiotic qualities to plants [8]. Additionally, as the farming of insects is expected to expand as sustainable ingredients for terrestrial and aquatic animals, this will also increase the availability of frass which can be viewed as a valuable resource. In particular, several studies report that using black soldier fly (*Hermetia illucens*) larvae (BSFL) frass as a soil amendment produced similar plant growth [9–11] or even better compared to those receiving synthetic fertilizers [12, 13]. In an aquaponics context, BSFL frass tea had no effect on either sweet banana chili pepper (*Capsicum annuum*) or sweetpotato (*Ipomea batatas*) slip production but did increase manganese and sugar, respectively, as well as consistently elevated water calcium, potassium, and phosphorus levels [5].

The type of system the plants are cultured in can also have a significant effect on productivity. While floating rafts and media-filled beds are some of the most common types, media is sometimes avoided due to the higher cost and concern that the accumulated debris will subsequently encourage anoxic zones and undesirable bacterial growth/toxins [3, 14]. However, there is increasing evidence indicating that leafy greens grow faster in media, as observed in lettuce (*Lactuca sativa*) [15], mint (*Mentha arvensis*) [18], spearmint (*Mentha spicata*) [17], lemongrass (*Cymbopogon citratus*), spring onion (*Allium fistulosum*) [18], and sweetpotato slips [5]. Research into this is still new, particularly for aquaponic plants grown in cooler waters.

The aim of this study was to compare the production, elemental composition, and chlorophyll content of collard greens when cultured in a floating raft or media-filled bed with or without BSFL frass additions.

2. Materials and Methods

2.1. Description of Aquaponic System. There were six identical aquaponic systems located under a greenhouse that was previously described [5]. Briefly, a 1000 L fish culture tank (175 cm deep, 160 cm wide × 43.1 height cm; polyethylene) emptied into a sump where water was then pumped (1 hp) into two separate plant culture beds that were identical in size/volume (78.7 cm length × 116.8 cm width × 40.6 cm height; 280 L capacity; polyethylene). However, one of the beds had a floating raft (60.9 cm length × 121.9 cm width × 5 cm thick; polyethylene foam) while the other bed contained fire-expanded lava rock that had a bell siphon, which created a periodic ebb and flows throughout the day, with a hydraulic retention time of approximately 5 min. All the pipes used were polyvinyl chloride (PVC; 3.91 cm diameter). The flow rates to the beds were adjusted to provide a similar flow to each plant bed, which was approximately 60 L/min.

2.2. Source of Plants, Fish, and Frass. Newly sprouted collard greens (3–5 mm) were purchased from a local nursery (Shell Ross, Pine Bluff, AR). The catfish (*Ictalurus punctatus*) juveniles were hatchery produced at the University of Arkansas at Pine Bluff and cultured on-site for several months. The fish were daily fed floating feeds designed for catfish (Rangen), and prior to adding to the aquaponic system, they were weighed and size graded. After fasting for 24 hr, a total of 20 catfish (128.4 ± 11.9 g) were added to each aquaponic system. After another day, they were hand fed twice each day to apparent satiation with floating feeds (Rangen; crude protein of 32%, crude lipid of 6%).

The frass used in this study was obtained from black soldier fly larvae (BSFL) produced in the laboratory according to Fischer and Romano [18]. Briefly, the BSFL were fed a combination of spent coffee grounds, dough, spoiled fish feeds, and a mixture of fruits/vegetables. After about a month, the frass was dried in a forced air oven (Despatch; LBB Series 2-12-3) at 100°C for two days and then ground into a fine powder with a hammer mill. The nutritional composition of the frass was measured at the Fayetteville Agricultural Diagnostic Laboratory at the University of Arkansas with inductively coupled plasma (ICP) analysis, and results were presented in Romano et al. [5]. The proximate composition of the BSFL frass was measured according to standard AOAC [19] methods and is presented in Table 1.

2.3. Experimental Design. In each of the six aquaponic systems, there were two plant culture beds: one with a floating raft that had three collard green plants, and the other bed was filled with media (lava rock) that had three collard green plants (Figure 1).

Prior to planting in the aquaponic system, the collard greens were removed from the soil, and the roots were gently rinsed/cleaned in standing water to remove as much soil as possible. In the floating raft, the collard greens were placed in a basket (5.5 cm diameter × 10.1 cm height) and held upright with lava rock. In the media, they were directly planted in the lava rock. In both plant culture beds (with or without media), the roots were able to touch the water.

Twice each week, 2.5 g of the BSFL frass was directly added to the sump. This amount was higher than previously used [5] and was based on some pilot studies conducted off-site. Once each week, all systems received 5 mL of iron chelate (Southern Ag; 5% soluble iron, 3.5% chelated iron; lignin sulfonate as a chelating agent) that was added to the sump. For the first week, the ammonia-N levels were measured each day from the culture tank. Afterward, the ammonia-N, nitrite-N, and nitrate-N levels were measured with an API master test kit. The water temperature, dissolved oxygen, and pH were measured with a digital multimeter probe (YSI Professional Plus).

Table 1: Proximate composition (% "as is" basis) of the black soldier fly (*Hermetia illucens*) larvae frass.

	Proximate composition
Dry matter	96.38
Crude protein	31.32
Crude lipid	8.55
Crude ash	23.71

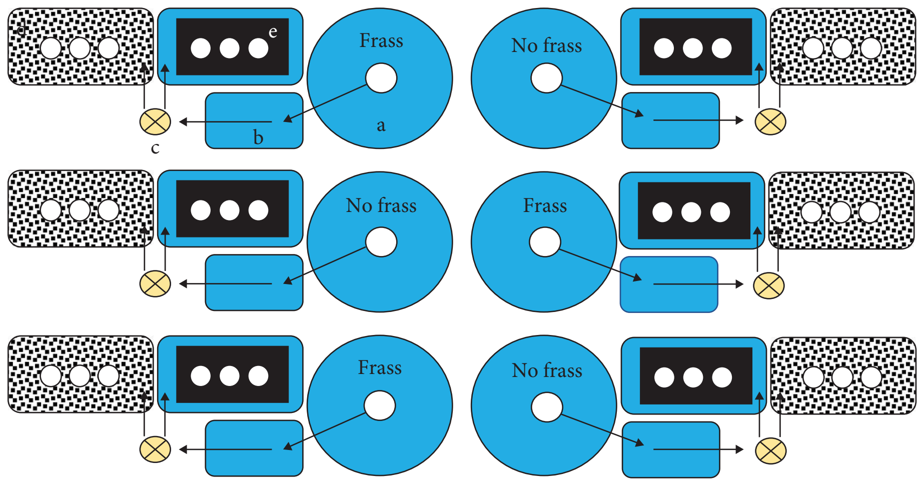

Figure 1: Layout of the six identical aquaponic systems and experimental design with a culture fish tank (1000 L) (a) where the water gravity fed into a sump (340 L) (b), that was pumped (c), to either the plant culture beds containing lava rock (media) (d) or floating raft (no media) (e) (280 L). The inlet to each plant bed was a rectangular pipe with numerous holes to help ensure an equal distribution of water. Three of these systems received weekly additions of black soldier fly (*Hermetia illucens*) larvae frass that was directly added to the sump while the other three received no frass supplementations.

2.4. Aquaponic Sampling. The collard greens were harvested three times during this study, which consisted of counting the harvestable leaves and their weights from each treatment. After the third harvest, the stem diameter was measured with a digital caliper, the plant height was measured with a ruler, and the entire plant was removed so the root lengths could also be measured.

2.5. Element Composition and Chlorophyll Content. From the first and last harvest, a subsample of these leaves was weighed (wet weight) and then dried in a forced air oven at 60°C for 24 hr when constant weight was achieved (dry weight). The wet and dried samples were used to calculate the moisture content. Next, the dried leaves (approx. 0.5 g) were digested for 30 min at 115°C after adding 4.0 mL HNO_3 (trace-metal-grade; 69%, Sigma-Aldrich). After digestion, 0.1 mL of H_2O_2 (30%) was added to 40 mL of Milli-Q water. Finally, the samples were measured with an inductively coupled plasma optimal emission spectrometer (Agilent 5800 VDV ICP-OES) according to AOAC 990.08 [19].

A subsample of leaves from the last harvest was taken, wrapped in aluminum foil, and kept at −20°C until analysis (within 3 weeks). The chlorophyll content of the leaves was measured in triplicate according to the dimethyl as described in Romano et al. [5]. Briefly, the leaf samples (approx. 0.1 g) were added to 1 mL of 80% ethanol, and then, this was left overnight at 4°C in the dark. After centrifuging the samples at 10,000 rpm for 10 min, 200 μL of the supernatant was measured on a 96-well plate at 664 nm and 647 nm to obtain the chlorophyll *a* and *b* content, respectively. The data were then divided by the weight of the sample to determine the amount of chlorophyll per gram.

2.6. Statistical Analysis. Data are presented as means ± SE, and prior to statistical analysis, the homogeneity of variance and normality was first confirmed. When with and without frass treatments were compared (water quality, water minerals, and values obtained from fish), an independent samples *t*-test was used. A two-way ANOVA was used to compare with or without media and with or without BSFL frass. Additionally, a one-way ANOVA was run among these four treatments, and if significant differences were found ($p < 0.05$), Duncan's post hoc test was used to identify the differences among treatments.

3. Results

3.1. Water Quality. The measured water quality parameters that included the dissolved oxygen, pH, hardness, dissolved nitrogen, and temperature were not significantly different among treatments (Table 2).

3.2. Fish Performance. Fish growth and feeding efficiency were not significantly different between treatments ($p > 0.05$).

Table 2: Mean (±SE) water quality characteristics over 10 weeks in aquaponic systems receiving black soldier fly (*Hermetia illucens*) larvae frass or none that was sampled from the sump.

Water quality	No frass	Frass	p-values
Dissolved oxygen (mg/L)	7.26 ± 0.24	7.14 ± 0.46	0.882
pH	7.36 ± 0.11	7.32 ± 0.18	0.743
Hardness (mg/L $CaCO_3$)	78.32 ± 0.44	83.21 ± 0.57	0.580
Ammonia-N (mg/L)	0.10 ± 0.05	0.15 ± 0.09	0.649
Nitrite-N (mg/L)	0.05 ± 0.00	0.05 ± 0.00	0.945
Nitrate-N (mg/L)	15.32 ± 0.78	18.72 ± 0.93	0.582
Temperature (°C)	19.15 ± 2.56	20.38 ± 2.86	0.825

No mortalities occurred in any of the tanks after 10 weeks of culture (Table 3).

3.3. Collard Green Production and Characteristics. The moisture content was significantly lower in the no media/no frass treatment compared to those in the other treatments (Table 4). The total weight was significantly highest ($p < 0.05$) in the media/frass treatment while the stem diameter was significantly highest in the media/no frass and media/frass treatments than those cultured without media (Table 4). The root length was significantly longest in the no media/no frass treatment while the height was significantly highest in the media treatments (Table 4). There was a significant media and frass effect on the moisture content, total weight, individual leaf weight, and height of the collard greens (Table 4). The root length and stem diameter were significantly influenced by the media while frass had a significant effect on the total count (Table 4).

3.4. Composition of Elements and Chlorophyll Content. Among the tested elements, zinc was significantly highest in the no media/no frass treatment, and moreover, there was a significant media, frass, and interactive effect (Table 5). The BSFL frass significantly elevated the manganese content, while no media significantly elevated the phosphorus content (Table 5). Based on the 2-way ANOVA, there was a significant media, frass, and interactive effect on the zinc content. Meanwhile, there was a significant frass and media effect on the manganese and phosphorus content, respectively (Table 5).

The chlorophyll a as well as the total chlorophyll content was significantly highest in the media/frass treatment compared to the no media/frass and no media/no frass treatments (Figure 2). The chlorophyll a and total chlorophyll content were significantly lowest in the no media/no frass treatment. For the chlorophyll b content, this was significantly lower in the no media/no frass treatment compared to the media treatments of with or without frass (Figure 2). There was a significant media ($p = 0.013$ and 0.010) and frass ($p = 0.001$ and 0.001) effect on the chlorophyll a and total chlorophyll content. The chlorophyll b content was significantly affected by the frass ($p = 0.001$) but not by media ($p = 0.160$). There were no interactive effects on the chlorophyll a, b, or total content ($p > 0.05$).

Table 3: Mean (±SE) growth performance, feeding efficiency, and survival of channel catfish (*Ictalurus punctatus*) juveniles cultured in an aquaponic system receiving black soldier fly (*Hermetia illucens*) larvae frass supplementations or none after 10 weeks.

	No frass	Frass	p-value
Final weight	327.0 ± 35.5	324.3 ± 16.2	0.949
Weight gain (%)	154.6 ± 47.9	152.6 ± 12.6	0.762
Total feed intake (kg)	5.60 ± 0.51	5.16 ± 0.32	0.512
FCR	1.37 ± 0.09	1.32 ± 0.07	0.709
Survival*	100.0 ± 0.0	100.0 ± 0.0	*

*Statistics on survival could not be performed due to no variability.

4. Discussion

The results indicate that a combination of BSFL frass additions as well as media was the best treatment for enhancing collard green production. Over the three harvests, the total weight was over threefold and fivefold greater in the media/frass treatment compared to the media/no frass and no media/no frass treatments, respectively. Moreover, both the frass and media had a significant effect on producing individual leaves that were heavier as well as minimizing chlorosis that occurred in the other treatments.

4.1. BSFL Frass. The nutritional composition of BSFL frass, and thus their potential effectiveness as an organic fertilizer, is influenced by the initial substrate provided to the BSFL. In general, substrates high in nitrogen will tend to also lead to higher nitrogen in the frass [18]. This is likely important to point out because not all BSFL frass products would have the same composition and such differences could lead to different outcomes. In an aquaponic context where dissolved nitrogen is often in abundance from the fish waste, it may be more beneficial to use less nitrogen-rich substrates, such as fruits and cereals, as compared to substrates derived from animals or spoiled fish feeds/plant proteins. In this study, even though the BSFL frass was produced on a mixture of high- and low-nitrogen substrates, nitrogen was the dominant nutrient. In fact, the nitrogen content of the frass was threefold higher or more than the other macronutrients that included phosphorus, potassium, and calcium. In the aquaponic system, however, the additions of BSFL frass had no significant effect on the dissolved nitrogen content or the other measured water quality parameters. In fact, ammonia-N and nitrite-N were often at undetectable levels while nitrate-N levels remained relatively low compared to several other aquaponic studies (e.g., [5, 20]).

Many of the productivity parameters that included total weight, total count, individual leaf weight, and height were significantly increased by BSFL frass additions. In fact, when comparing the final total weight from nine plants, this was fivefold higher in the system receiving BSFL frass. For example, from nine plants, almost 6 kilos of sellable collard greens were obtained in the media/frass treatment compared to 1.6 kilos in the media/no frass treatment. This difference was attributed to only adding 5 g of BSFL frass/week, and

Table 4: Production parameters of collard greens aquaponically grown with or without media and with or without black soldier fly (*Hermetia illucens*) frass after 10 weeks.

	No media/no frass	No media/frass	Media/no frass	Media/frass	SEM	Media	Frass	Interaction
Moisture (%)	87.95[a]	89.61[b]	90.64[b]	90.75[b]	0.31	**0.001**[***]	**0.047**[***]	0.073
Total weight (g)*	217[c]	1290[b]	546[c]	1995[a]	167	**0.032**[***]	**0.001**[***]	0.373
Total count*	43[a]	68[a]	52[a]	71[a]	9.41	0.544	**0.048**[***]	0.785
Individual leaf weight (g)	4.89[c]	19.91[b]	9.58[c]	28.11[a]	1.51	**0.001**[***]	**0.005**[***]	0.333
Stem (cm)	9.72[b]	11.63[b]	17.66[a]	18.39[a]	1.19	**0.001**[***]	0.329	0.652
Root (cm)	49.44[a]	44.67[ab]	21.00[b]	34.88[ab]	5.91	**0.038**[***]	0.598	0.279
Height (cm)	15.56[b]	18.22[b]	24.44[a]	27.56[a]	1.10	**0.001**[***]	**0.035**[***]	0.849
Total count**	130	204	156	214	n/a	n/a	n/a	n/a
Total weight (g)**	653	3870	1638	5985	n/a	n/a	n/a	n/a

*mean among three plants from three harvests. **total from three plants from three harvests. n/a = sum of nine plants (3/replicate) in each treatment and thus no replicates to conduct statistical analysis. ***bold font indicates significant differences.

Table 5: Mean (±SE) composition of elements (mg/kg on "as is basis") in collard greens aquaponically grown with or without media and with or without black soldier fly (*Hermetia illucens*) frass after 10 weeks.

	No media/no frass	No media frass	Media/no frass	Media/frass	SEM	Media	Frass	Interaction
Phosphorus	192.3	173.8	147.0	167.0	10.9	**0.045**[*]	0.944	0.117
Sodium	15325.7	12171.5	10909.6	9486.3	1315.6	0.059	0.194	0.606
Calcium	2639.9	2353.7	2576.0	2363.8	275.2	0.932	0.435	0.906
Sulfur	53.3	37.7	33.5	32.3	5.2	0.062	0.188	0.254
Iron	90.9	44.8	1080.0	178.2	45.0	0.857	0.278	0.395
Manganese	93.3	445.6	122.2	457.1	86.9	0.861	**0.016**[*]	0.940
Zinc	1530.6	498.1	443.2	594.3	168.8	**0.034**[*]	**0.021**[*]	**0.009**[*]
Strontium	763.1	659.8	549.9	676.8	90.8	0.321	0.902	0.250
Aluminum	382.8	295.1	283.6	292.0	27.5	0.144	0.244	0.167

*bold font indicates significant differences.

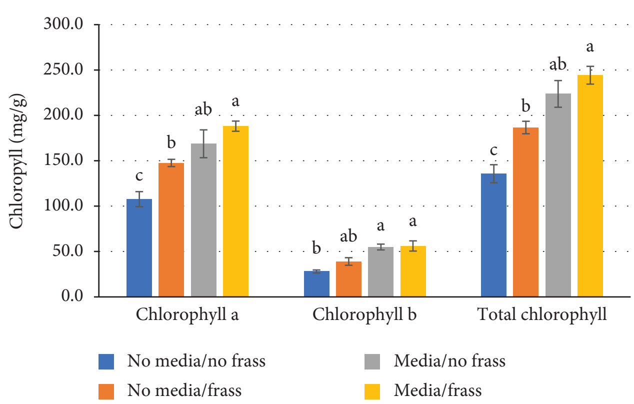

Figure 2: Mean (±SE) chlorophyll a, b and total chlorophyll content (mg/g wet weight) of collard greens with or without black soldier fly (*Hermetia illucens*) frass after 10 weeks.

such a remarkable improvement could be attributed to the BSFL frass containing a mixture of both essential macro- and micronutrients. In fact, when BSFL frass was converted into "tea," many of the micronutrients were undetectable and, moreover, had no significant effect on sweetpotato slip or sweet banana chili pepper production [5]. Similarly, when culturing sweetpotato slips in soil, the use of BSFL frass tea was far inferior to BSFL frass [11]. Therefore, it is recommended that direct additions of BSFL frass are made, but ways to potentially mineralize the frass may be a worthwhile research direction.

Besides growth, the other obvious visual difference between plants receiving BSFL frass and not was some "chlorosis," based on the yellowing of the collard green leaves but with green veins. Chlorosis is attributed to a lower chlorophyll content subsequently leading to inhibited growth and other processes in plants [6, 7, 21]. In order to quantify chlorosis in this study, the chlorophyll content of the collard green leaves was measured from the last sampling. It was indeed found that collard greens not receiving BSFL frass had significantly lower chlorophyll a, b, and $a + b$ content. Chlorosis is often attributed to deficiencies in Fe

[21] as well as Mg and Ca in aquaponic systems [4]. Although iron chelate was added to all systems each week, the added amounts were equal. Therefore, it seems possible that because the BSFL frass contains various minerals, including Fe, Mg, Ca, this might have contributed to minimizing chlorosis and enhancing growth. Interestingly, however, among the tested elements, Mn was the only element that was significantly increased in the collard green leaves by BSFL frass. This was similarly reported in sweetpotato slips where adding BSFL frass significantly elevated Mn in the leaves compared to those receiving synthetic fertilizers when grown in soil [11].

It is important to note that the amount of added BSFL frass to the aquaponic system had no significant effect on catfish growth, feeding efficiency, or survival. This could be due to the BSFL having no negative impact on water quality and is consistent with the findings of [5].

4.2. Use of Media. Similar to the BSFL frass, the use of a media-filled bed significantly enhanced collard green production based on weight as well as the stem diameter and overall height, compared to those cultured in a floating raft. Even visually, it was clear that collard greens grown in media were far more robust compared to those in the floating raft, which is consistent with other reports on leafy green growth in aquaponic systems [15, 18]; Knaus et al. 2020 [5, 18]. It is known that flow rates can greatly influence plant growth in an aquaponic system [22], but the flow rates between the two plant beds were similar in this study. The better growth might be related to minerals being more accessible as a result of organic debris accumulation in the media-filled bed and subsequent bacterial mineralization, but this requires more investigation. It is perhaps important to note that the plants grown in media or floating rafts were sharing the same water and thus were in competition for the same nutrients. It is therefore conceivable that if plants in each system were not sharing the same water, the results could be different; however, other studies have found that media-filled beds support more leafy growth when the different beds were separated [15, 18]; Knaus et al. 2020.

Although the enhanced growth of the collard greens would imply more nutrients, P and Zn were the only measured nutrients in the collard green leaves that were significantly altered by the system, and both were higher in the floating raft treatment. Other studies showed no mineral content difference in plants cultured in a floating raft versus a media-filled bed [5, 18]. Nevertheless, this finding of higher elements was unexpected considering most of the collard greens cultured in the floating raft (with or without BSFL frass) exhibited some degree of chlorosis, which was quantified based on significantly lower chlorophyll content. Further research should explore the cause(s) for chlorosis because this may influence willingness to buy.

5. Conclusions

Among the tested treatment combinations, the media/frass treatment led to a ninefold increase in weight compared to the worst treatment of no media/no frass. The substantially higher growth was likely due to a greater accessibility to nutrients that was provided by the BSFL frass and possibly increased biomineralization in the media. In the case of the BSFL frass, this contained various essential nutrients that were added to the system. In the case of media, this may have accumulated more organic debris and facilitated biomineralization. However, more research is certainly needed to better understand the effects of BSFL frass and media have on aquaponic plant growth and composition. Nevertheless, the combined culture of collard greens in media with BSFL frass supplementations could be recommended to enhance leafy growth production. There could also be implications for willingness to buy considering BSFL frass and media-created leaves were larger and darker in color. Further research on different frass sources, whether from different insect species or substrates, could be worthwhile to improve aquaponic production and sustainability.

Data Availability

The data can be made available from the corresponding author upon reasonable request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

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