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Partially replacing synthetic fertilizer with black soldier fly (*Hermetia illucens*) larvae frass enhances kale (*Brassica oleracea* var. *sabellica*) production

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Abstract

A by-product of insect farming is 'frass', which is the leftover excrement that can be high in essential minerals. The frass of black soldier fly (*Hermetia illucens*) larvae (BSFL) is increasingly gaining interest as a soil amendment, but information is limited regarding the feasibility of partially replacing synthetic fertilizers. In this 5-week study, newly-sprouted kale plants were grown with weekly applications of a synthetic fertilizer (control), diluted synthetic fertilizer combined with BSFL frass as a one-time application (½ fert frass) and only BSFL frass as a one-time application (frass). Results showed that the ½ fert frass treatment led to significantly higher biomass of kale than the other treatments, while the stem diameter and height was significantly higher than the control. Potassium and magnesium in kale leaves were significantly lower in the control compared the ½ fert frass treatment while manganese, sodium and zinc were significantly higher in the frass treatment than the control. Iron was significantly highest in the control than the other treatments, but chlorophyll was significantly higher in the ½ fert frass treatment compared to the frass treatment. Among the monosaccharides, mannose was significantly elevated in the leaves of kale grown with only frass. Results indicate that not only can BSFL frass partially replace synthetic fertilizer, but actually showed a growth benefit compared to the sole use of either synthetic fertilizer or BSFL frass.

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Introduction

Insect farming is gaining interest to sustainably supply the livestock and aquaculture industry with high quality protein and lipids. Among the various insects, black soldier fly (*Hermetia illucens*) larvae (BSFL) are especially attractive to farm due to being able to thrive on various organic waste streams, are not a nuisance species, and when included in the diets of livestock and fish, there have been important benefits. Some of these benefits include improvements to immunity in various animal species[1], protecting the intestine in fish from inflammation[2,3], as well as enhancing broiler growth and the quality of eggs from laying hens[4]. With research demonstrating the various benefits to the inclusion of BSFL in animal and fish diets, it is likely that the BSFL industry will continue to grow.

A major by-product from BSFL farming is the leftover 'frass', which mainly consists of their excrement as well as chitin due to the shedding of their exoskeleton as the larvae develop[5]. Rather than viewing frass as a challenge for responsible disposal, BSFL frass is increasingly being investigated as a potential soil amendment for terrestrial farming. To date, the results from applications of BSFL to plant productivity have been remarkably promising. When used on plants, BSFL frass was either comparable with synthetic fertilizers[6−8] or provided superior production[9−12]. Some of the causes were believed to be from improvements to soil quality, a higher quantity of beneficial microorganisms and/or the presence of various accessible nutrients[5]. Moreover, compared with the frass of other insects, BSFL frass has substantially more nitrogen, potassium and sulfur, has similar amounts of phosphorus and magnesium and the lowest content of phytotoxic compounds and pathogens[11]. The bioconversion of organic waste into BSFL frass is also relatively fast compared to other biodegradation methods where, depending on the initial substrate and stocking density, this can take one to three weeks[5].

It is known that synthetic fertilizers have a higher nutrient accessibility to plants compared with organic fertilizers but there are some drawbacks to their overuse. Some of these include being more prone to run-off that damages natural waterways, a deterioration of soil quality in the long run as well as being less sustainable[13]. Moreover, there are currently supply disruptions with the synthetic fertilizer trade and thus it may become necessary to find feasible alternatives as at least partial replacements. To the best of our knowledge, there is only one study that examined the combined effects of BSFL frass with fertilizer and this reduced the heavy metal content in rice when used with a selenium foliar spray[14]. On the other hand, there are numerous reports that the combined use of organic and inorganic fertilizers can enhance plant production, which is often attributed to improved soil properties[15−18]. However, it is unclear whether BSFL frass can partially replace a multimineral synthetic fertilizer or may even lead to fertilizer burn.

The aim of this study was to compare the growth, mineral, chlorophyll, and monosaccharide composition of kale (*Brassica* *oleracea* var. *sabellica*) when receiving weekly applications of a multimineral synthetic fertilizer, versus a partial (50%) replacement of synthetic fertilizer with BSFL frass and only BSFL frass as a one-time top dressing.

Materials and methods

Source of plants and black soldier fly larvae frass

Kale seeds (Ferry-Morse®, USA) were purchased and planted in germination trays that were located under a greenhouse at University of Arkansas at Pine Bluff. The seeds were watered daily and after eight days, the seedlings emerged. Three days later, when the plants were approximately 4 cm in height, they were transferred into plastic pots (29 cm × 29 cm × 28 cm; 0.024 m^3), filled with approximately 1.2 kg of peat moss (as is basis) and then each pot was irrigated. Each pot had numerous holes on the bottom to self-drain as well as a plastic saucer to catch any excess water.

The BSFL frass was produced from a combination of household food waste, fruit peels, spoiled vegetables, stale bread, and spoiled animal feeds and described in detail in Romano et al.[12]. The BSFL frass was processed by drying at 100 °C for 48 h and hammer milled into a fine powder. The nitrogen-phosphorus-potassium (NPK) of the BSFL frass was 4.6-2.5-2.9, while the calcium and magnesium content were 5.28% and 0.44%, respectively. A more detailed report of the mineral composition is provided in Romano et al.[12].

The commercial synthetic fertilizer (Sta-Green) was purchased and was chosen based on having a balanced NPK value (18-18-21) as well as being recommended for vegetables.

Experiment design

The planting pots were arranged in three rows of three. After the seedlings were planted, the treatments were allocated in a randomized block design to help minimize any bias with their position to light or temperature.

Once the treatments were allocated and labelled, the control received the synthetic fertilizer, which was diluted with water (10.4 g into 3.78 L) according to the manufacturer's instructions. The control subsequently received weekly applications of the synthetic fertilizer. The ½ fert frass treatment received weekly applications of diluted fertilizer, which was 5.2 g into 3.78 L, as well as a one-time top dressing of BSFL frass of 8.8 g (equivalent to 1 cup). The ½ fert frass treatment received diluted synthetic fertilizer on the same day as the BSFL frass application. The frass treatment only received a one-time top dress of BSFL of 8.8 g (as above). The amount of frass added was equivalent to 0.37 kg/m^3.

Each day the pots were watered with collected rainwater, except for one/week the control and ½ fert frass treatments received synthetic fertilizer applications.

After five weeks the final height was measured along with the stem diameter using a digital caliper. Kale was then cut at the stem and weighed on a digital scale for the final biomass. Half the leaves were then wrapped in aluminum foil and stored at −20 °C in air-tight plastic bags and measured for chlorophyll within one week. The other half of the leaves were dried in an oven at 65 °C to calculate the moisture and then analyzed for the mineral and sugar composition.

Mineral composition

The dried kale leaves from each replicate/treatment were ground into a fine powder using a coffee grinder and measured for the calcium, potassium, magnesium, iron, manganese, sodium and zinc content using a flame atomic absorption spectrophotometer (AAS) (iCE 3000 series, Thermo Scientific, Santa Clara, CA, USA) that was equipped with a deuterium lamp for background correction according to methods described in detail in Romano et al.[12]. The total phosphorus level was not high enough for the AAS to detect, and therefore, kale leaf samples were measured using the persulfate digestion method (HACH method 8190).

Chlorophyll content

The chlorophyll content of the kale leaves was measured in triplicate according to the dimethyl formamide method as described in Sibley et al.[19]. The leaves were weighed ~0.1g, and then 1 mL of 80% ethanol was added, which was left overnight in the dark at 4 °C. After 24 h, the samples were centrifuged at 10,000 rpm for 10 min and then 200 μL of the supernatant was transferred into a 96-well microplate that was read at 664 nm and 647 nm. The absorbance for each wavelength were used to calculate the chlorophyll content[20,21].

Monosaccharide composition

The monosaccharide composition that included xylose, arabinose, fucose, ribose, mannose, glucose and galactose of the kale leaves were measured in triplicate at the Agriculture Experiment Station Chemical Laboratories, University of Missouri-Columbia according to those described in detail in Oxley et al.[22].

Results

Kale growth

After five weeks, the weight, stem diameter and height of the kale in the control (weekly applications of a synthetic fertilizer) was significantly less than the ½ fert frass treatment. While weight was significantly lower for kale grown with only BSFL frass compared to the ½ fert frass treatment, there was no significant difference in the stem diameter or height (Fig. 1).

Mineral composition

Among the macronutrients, potassium and magnesium were both significantly lower in the control as compared to the ½ fert frass treatment (Table 1). Moreover, magnesium was significantly lower in the control than the frass treatment. Among the micronutrients, iron was significantly higher in the control than the other frass treatments. On the other hand, manganese, sodium, and zinc were significantly higher in the frass treatment compared to the control (Table 1).

Moisture, chlorophyll and monosaccharide content

There was no significant difference in moisture content among the treatments (Table 2). Chlorophyll was significantly higher in the ½ fert frass treatment compared to the frass treatment (Table 2). Among the tested monosaccharides, only mannose was significantly affected by the treatment, which was lower in the control compared to those grown with only BSFL frass (Table 2).

Discussion

The results of this study demonstrated that not only could BSFL frass partially replace the tested synthetic fertilizer, but such a replacement actually led to significantly better production compared to either the sole use of either the synthetic

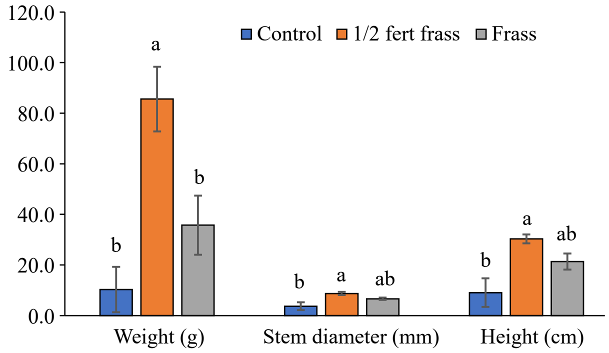

Fig. 1 Mean (± SE) growth of kale when receiving weekly applications of a synthetic fertilizer (control), diluted synthetic fertilizer with black soldier fly larvae (BSFL) frass as a one-time application (½ fert frass) and only BSFL frass as a one-time application (Frass). Different letters indicate significant differences ($p < 0.05$).

Table 1. Mean (± SE) mineral composition (mg/g) of kale when receiving weekly applications of a synthetic fertilizer (control), diluted synthetic fertilizer with black soldier fly larvae (BSFL) frass as a one-time application (½ fert frass) and only BSFL frass as a one-time application (frass).

	Control	½fert frass	Frass
Macronutrients			
Phosphorus	1.26 ± 0.28^{a}	1.50 ± 0.17^{a}	1.71 ± 0.07^{a}
Calcium	4.02 ± 0.05^{a}	4.24 ± 0.27^{a}	3.91 ± 0.26^{a}
Potassium	16.19 ± 1.76^{b}	35.67 ± 4.00^{a}	30.37 ± 5.19^{ab}
Magnesium	2.75 ± 0.10^{b}	3.52 ± 0.26^{a}	3.68 ± 0.11^{a}
Micronutrients			
Iron	0.36 ± 0.03^{a}	0.10 ± 0.01^{b}	0.15 ± 0.07^{b}
Manganese	0.07 ± 0.00^{b}	0.05 ± 0.00^{b}	0.09 ± 0.01^{a}
Sodium	1.32 ± 0.04^{b}	1.40 ± 0.01^{a}	1.44 ± 0.01^{a}
Zinc	0.05 ± 0.01^{b}	0.11 ± 0.02^{b}	0.39 ± 0.06^{a}

Different superscripted letters in the same row indicate significant differences ($p < 0.05$).

Table 2. Mean (± SE) moisture (%), chlorophyll (mg/g), and monosaccharide composition (g/100 g) of kale when receiving weekly applications of a synthetic fertilizer (control), diluted synthetic fertilizer with black soldier fly larvae (BSFL) frass as a one-time application (½ fert frass) and only BSFL frass as a one-time application (frass).

	Control	½fert frass	Frass
Moisture (%)	80.77 ± 1.40	85.59 ± 0.53	84.12 ± 1.80
Chlorophyll	1.31 ± 0.20^{ab}	1.62 ± 0.07^{a}	1.02 ± 0.02^{b}
Monosaccharides			
Xylose	0.35 ± 0.08	0.46 ± 0.08	0.38 ± 0.05
Arabinose	0.56 ± 0.05	0.57 ± 0.02	0.51 ± 0.03
Fucose	0.16 ± 0.03	0.15 ± 0.01	0.14 ± 0.01
Mannose	0.19 ± 0.01^{b}	0.26 ± 0.02^{ab}	0.27 ± 0.02^{a}
Glucose	5.90 ± 0.55	3.87 ± 0.31	5.07 ± 0.84
Galactose	0.76 ± 0.02	0.81 ± 0.02	0.83 ± 0.03
Total	7.92 ± 0.54	6.12 ± 0.02	7.20 ± 0.03

Different superscripted letters in the same row indicate significant differences ($p < 0.05$).

fertilizer or BSFL frass. This could indicate an additive or synergistic effect between the synthetic fertilizer and BSFL frass or the fact that the ½ fert frass treatment received a double dose at the beginning of the study. Similarly, a combination of inorganic and organic fertilizers (in the form of animal manure) led to better plant production, compared to the sole use of either fertilizer type[15−18]. Such findings were often suggested to be from, at least in part, to better soil qualities, particularly soil organic matter[15−18]. Moreover, several recent studies have demonstrated that chitin-rich BSFL exuviae, which is present in BSFL frass, can enhance plant health and/or production by encouraging the abundance and duration of beneficial bacteria in the soil[23−25]. More research is required to better characterize this relationship to optimize production as well as improve sustainable farming practices.

Another interesting finding was that the sole use of BSFL frass as a one-time top dressing led to kale outperforming those receiving weekly applications of a synthetic fertilizer. This finding was not anticipated based on our previous findings on basil and jalapeno plants in which a one-time application of BSFL frass eventually led to inferior growth (unpublished results). In those studies, however, a 16-fold lower dose of BSFL frass was applied, compared to this study, and it was believed that a higher dose may be more effective. Therefore, in this study, the BSFL frass was applied at a 16-fold higher dose to test this hypothesis but also to determine if such high levels would harm the plants, such as causing burning of the leaves. Indeed, Setti et al.[9] found that BSFL frass applications can become excessive (from 30% up to 40%) to basil, tomatoes and baby lettuce, based on reduced growth. Setti et al.[9] suggested that BSFL frass applications can eventually become excessive by causing phytotoxicity to the plants. In this study, a total of 8.8 g of heat-treated and hammer milled BSFL frass was added to approximately 1.2 kg of soil, which was less than 1% to the overall weight of the substrate, and substantially less than those of Setti et al.[9]. It seems possible that the superior growth of kale in the ½ fert frass treatment, compared to the other treatments, was due to minimizing the chances of causing phytotoxicity as well as causing nutrient(s) deficiency.

In order to potentially provide additional insight regarding nutrient limitations as well as nutritional value to humans, plant tissue analysis for minerals was performed at the end of this study. In this study, all the tested macronutrients were either similar among treatments or significantly higher in the BSFL frass treatments. Potassium was significantly higher in the ½ fert frass treatment compared to the control while magnesium was significantly higher in the ½ fert frass and BSFL frass treatments compared with the control. This indicates that although the macronutrients within BSFL frass were likely slower releasing than synthetic forms, the frass was provided in sufficient amounts for kale growth. Moreover, among the tested micronutrients, these were all significantly higher in kale leaves when grown only with BSFL frass with the exception of iron. Chlorophyll synthesis in plants is mediated by iron[26], but the relationship between iron in kale leaves and the chlorophyll content was not evident in this study.

Monosaccharides have many functions in plants that include their roles in plant growth, structure as well as influences on taste and health for human consumers[27]. Among the tested monosaccharides, only mannose was significantly altered by the fertilizer treatment, which was higher in the BSFL frass treatment. The cause for this is unclear and could be worthwhile to explore further considering evidence showing various benefits to human health including having anti-inflammatory and anti-tumor properties[28].

An advantage to farming BSFL is their ability to thrive on various waste streams and thus can become an integral part of a circular economy. In this study, it was shown that the initial substrate provided to the BSFL, which was a mixture of locally available organic waste, was subsequently valorized as beneficial organic fertilizer. It has been shown that while the carbon and nitrogen content of BSFL frass is relatively stable, phosphorus, potassium and various micronutrients can vary substantially based on the provided substrate[5]. Therefore, the type of provided substrate may influence their efficacy as an organic fertilizer while another potential factor may include whether or not the frass was raw or processed, such as by drying and/or being ground up. Further research into these areas could be worthwhile towards optimizing the composition and processing methods of BSFL frass for different plant species.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Koutsos E, Modica B, Freel T. 2022. Immunomodulatory potential of black soldier fly larvae: Applications beyond nutrition in animal feeding programs. *Translational Animal Science* 6:txac084
2. Kumar V, Fawole FJ, Romano N, Hossain MS, Labh SN, et al. 2021. Insect (black soldier fly, *Hermetia illucens*) meal supplementation prevents the soybean meal-induced intestinal enteritis in rainbow trout and health benefits of using insect oil. *Fish & Shellfish Immunology* 109:116−24
3. Agbohessou PS, Mandiki SNM, Mbondo Biyong SR, Cornet V, Nguyen TM, et al. 2022. Intestinal histopathology and immune responses following *Escherichia coli* lipopolysaccharide challenge in Nile tilapia fed enriched black soldier fly larval (BSF) meal supplemented with chitinase. *Fish & Shellfish Immunology* 128:620−33
4. Dörper A, Veldkamp T, Dicke M. 2021. Use of black soldier fly and house fly in feed to promote sustainable poultry production. *Journal of Insects as Food and Feed* 7:761−80
5. Lopes IG, Yong JWH, Lalander C. 2022. Frass derived from black soldier fly larvae treatment of biodegradable wastes: A critical review and future perspectives. *Waste Management* 142:65−76
6. Klammsteiner T, Turan V, Fernández-Delgado Juárez M, Oberegger S, et al. 2020. Suitability of black soldier fly frass as soil amendment and implication for organic waste hygienization. *Agronomy* 10:1578
7. Chirere TES, Khalil S, Lalander C. 2021. Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from food waste and faeces. *Journal of Insects as Food and Feed* 7:457−69
8. Chiam Z, Lee JTE, Tan JKN, Song S, Arora S, et al. 2021. Evaluating the potential of okara-derived black soldier fly larval frass as a soil amendment. *Journal of Environmental Management* 286:112163
9. Setti L, Francia E, Pulvirenti A, Gigliano S, Zaccardelli M, et al. 2019. Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. *Waste Management* 95:278−88
10. Agustiyani D, Agandi R, Arinafril, Nugroho AA, Antonius S. 2021. The effect of application of compost and frass from black soldier fly larvae (*Hermetia illucens* L.) on growth of pakchoi (*Brassica rapa* L.). *IOP Conference Series: Earth and Environmental Science* 762:012036
11. Beesigamukama D, Mochoge B, Korir NK, Fiaboe KKM, Nakimbugwe D, et al. 2020. Exploring black soldier fly frass as novel fertilizer for improved growth, yield, and nitrogen use efficiency of maize under field conditions. *Frontiers in Plant Science* 11:574592
12. Romano N, Fischer H, Powell A, Sinha AK, Islam S, et al. 2022. Applications of black soldier fly (*Hermetia illucens*) larvae frass on sweetpotato slip production, mineral content and benefit-cost analysis. *Agronomy* 12:928
13. Shaviv A. 2001. Advances in controlled-release fertilizers. *Advances In Agronomy* 71:1−49
14. Geng W, Zhao Y, Mao Z, Wang X, Wu N, et al. 2022. The effects of combined use of black soldier fly larvae frass fertilizer with exogenous selenium on rice growth and accumulation of heavy metals. *Journal of Soil Science and Plant Nutrition* 22:5133−43
15. Basu M, Bhadoria PBS, Mahapatra SC. 2008. Growth, nitrogen fixation, yield and kernel quality of peanut in response to lime, organic and inorganic fertilizer levels. *Bioresource Technology* 99:4675−83
16. Zhang F, Xu P, Tang S, Chen J, Xie K, et al. 2008. Effects of chemical fertilizer and composts produced from chicken, swine and cattle manures on yields and quality of leaf vegetables. *Chinese Agricultural Science Bulletin* 24:283−86
17. Chen X, Cui Z, Fan M, Vitousek P, Zhao M, et al. 2014. Producing more grain with lower environmental costs. *Nature* 514:486−89
18. Vo MH, Wang CH. 2015. Effects of manure composts and their combination with inorganic fertilizer on acid soil properties and the growth of muskmelon (*Cucumis melo* L.). *Compost Science & Utilization* 23:117−27
19. Sibley JL, Eakes DJ, Gilliam CH, Keever GJ, Dozier WA, et al. 1996. Foliar SPAD-502 meter values, nitrogen levels, and extractable chlorophyll for red maple selections. *HortScience* 31:468−70
20. Aono M, Kubo A, Saji H, Tanaka K, Kondo N. 1993. Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tabacum* with high chloroplastic glutathione reductase activity. *Plant and Cell Physiology* 34:129−35
21. Inskeep WP, Bloom PR. 1985. Extinction coefficients of chlorophyll *a* and *b* in *N,N*-dimethylformamide and 80% acetone. *Plant Physiology* 77:483−85
22. Oxley D, Currie G, Bacic A. 2006. Monosaccharide composition analysis: Alditol acetates. *Cold Spring Harbor Protocols* 2006(1):4246
23. Barragán-Fonseca KY, Nurfikari A, van de Zande EM, Wantulla M, van Loon JJA, et al. 2022. Insect frass and exuviae to promote plant growth and health. *Trends in Plant Science* 27:646−54
24. Wantulla M, van Loon JJA, Dicke M. 2023. Soil amendment with insect exuviae causes species-specific changes in the rhizosphere bacterial community of cabbage plants. *Applied Soil Ecology* 188:104854
25. Wantulla M, van Zadelhoff K, van Loon JJA, Dicke M. 2023. The potential of soil amendment with insect exuviae and frass to control the cabbage root fly. *Journal of Applied Entomology* 147:181−91
26. Li J, Cao X, Jia X, Liu L, Cao H, et al. 2021. Iron deficiency leads to chlorosis through impacting chlorophyll synthesis and nitrogen metabolism in *Areca catechu* L. *Frontiers in Plant Science* 12:710093
27. Taiz L, Zeiger E. 2006. *Plant Physiology*. 4th Edition. Sunderland, MA, USA: Sinauer Associates, Inc.
28. Nan F, Sun Y, Liang H, Zhou J, Ma X, et al. 2022. Mannose: A sweet option in the treatment of cancer and inflammation. *Frontiers in Pharmacology* 13:877543