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# Black Soldier Fly Larva *(Hermetia illucens)* Frass vs. Red Wiggler *(Eisenia fetida)* Castings on *(Capsicum annum)* "Early Jalapeno" Seedling Growth.

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Abstract

Black soldier fly larvae (BSFL) *Hermetia illucens* have shown some promising signs in their ability to rapidly process organic waste into a usable organic frass input. However, little is known about the effectiveness of the frass, when compared to biofertilizers produced by other organic waste consumers, such as, red wiggler worms (RW) *Eisenia fetida*. This study compared the waste consumption rate, compost nutrient concentration, and compost application by BSFL and RW on jalapeno seedlings. The BSFL were shown to consume organic waste at a clearly higher rate than RW, while producing compost with higher N, P, K nutrient concentrations. Furthermore, an application test showed BSFL compost generating more seedling growth than a control with no biofertilizer. However, with no additional maturing processes the BSFL compost was not as effective for seedling growth, when compared to its RW biofertilizer counterpart.

*Keywords: Hermetia illucens*, *Eisenia fetida*, *Capsicum annum*, biofertilizer, waste conversion

## Introduction

Global population has seen super exponential growth rates during modern times (Murphy, 1994). Amazingly humans have, for the most part, been able to keep up with our sharp spike in total caloric needs. We are now in a position where supporting our current population requires us to sustain these elevated yields, with a predicted need to increase production as the population continues to grow. Simultaneously, these advancements in production capacity have led to undeniable external costs such as soil degradation, depletion of biodiversity, elevated greenhouse gases and polluted water sources (Hazell & Ramasamy, 1993).

While it is understatedly unreasonable to expect one magic fix all, looking for dynamic solutions which can address multiple problems would be a best-case scenario. Current waste management practices do little to actively reintegrate organic resources back into a production

cycle. This has prompted interest in establishing effective methods of repurposing waste in a cyclical manner.

Utilizing BSFL has shown to be a cost effective and environmentally friendly method of processing organic waste. However, although some claim that the frass byproduct could be used as an organic biofertilizer, there is little conclusive evidence indicating its efficacy. Using vermicompost (worm castings) as a known comparative standard, we can help determine whether BSFL can produce a high-quality biofertilizer.

The goal of this investigation is to compare the waste consumption rate, resultant compost nutrient content and compost application results of BSFL frass to that of red wiggler (RW) castings and a biofertilizer-less control. Additionally, microbial impact on plant growth will be looked at for each respective compost.

## Study Organisms

### Red wiggler worm (*Eisenia fetida*)

Most of the literature on organic waste composting through earthworms has been centered on the species *Eisenia fetida*. The interest in RW is primarily due to their digestive process which has displayed the ability to produce a stable and effective biofertilizer (Adhikary, 2012) (Fig. 1). In addition to simply converting waste to biofertilizer, vermicompost has shown the ability to provide several other soil health and plant growth promoting properties.

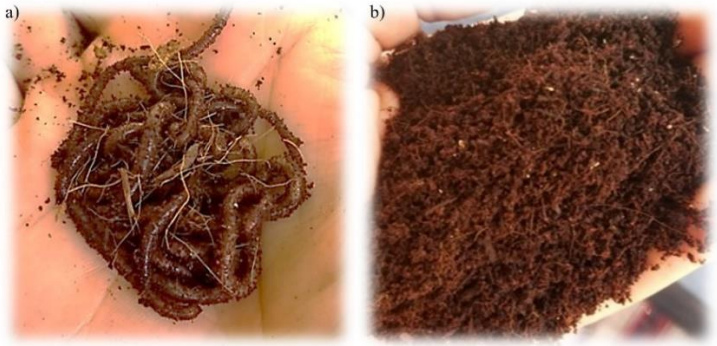


**Figure 1.** Composting worm species *Eisenia fetida* a), and its fecal castings byproduct (vermicompost) b).

**vermicompost nutrient content and yields.** Given the importance of plant available nutrients for plant growth, highlighting the nutrient reclamation ability of vermicompost is vital in showcasing its viability as a waste recycling option. By reducing organic C content of waste via biomass conversion and microbial respiration, concentrations of Total Kjeldahl Nitrogen (TKN) and plant available P can substantially increase in RW fecal byproducts. TKN, which is

the sum of organic nitrogen and ammonia, can be used to represent, the vital and often limiting, potential plant available N. To that end, Nath and Singh (2016) documented an increase of TKN content from 7.15 g/kg to 15.43 g/kg after vermicomposting buffalo excreta. These increases in TKN concentrations, while subsequently decreasing C through bioaccumulation will lower C/N ratios in vermicompost which can often imply compost stability. Unsurprisingly, elevating nutrient levels with vermicompost ultimately increases yields, for a variety of crops. For example, Yang et al. (2015) has shown that vermicompost application on greenhouse tomatoes outperformed manure compost and chemical fertilizer.

**vermicompost microbial activity.** Earthworms have a diverse microflora in their gut. By secreting digested organic matter, they effectively populate the soil with this diverse microbial activity (Edwards & Bohlen, 1996). This increase in microbial activity can help release nutrients into the soil solution, making them available to plants. Furthermore, plant hormones like gibberellin, cytokinin and auxin are produced via increased microbial metabolic activity, aiding in a plant's growth (Atiyeh et al., 2002). Lastly, vermicompost microbes can help control plant pathogens. Rivera et al. (2004) found that applications of vermicompost on tomato seedlings helped limit the impact of the *Rhizoctonia solani* pathogen responsible for damping off.

**vermicompost physical & chemical properties.** The unique digestive system of earthworms uses their intestinal walls to grind down ingested organic matter (Edwards & Bohlen, 1996). This method of digestion results in castings with a particularly large specific surface area (SSA) and subsequently an extremely high cation exchange capacity (CEC). Lastly, vermicompost has proven to help soil aggregation (soil molecules bound together through chemical and biological activity) (Aksakal et al., 2015).

## Black Soldier Fly Larvae (BSFL) *Hermetia illucens*

BSFL have shown a remarkable ability to quickly consume large quantities of waste. This ability has garnered interest in them as a potential tool. Additionally, BSFL makes for a protein-rich organism, with bioconversion rates of 40-45% protein, while also generating an organic frass byproduct (Fig. 2). As such, BSFL provides a prospective cost-effective and environmentally friendly waste management tool of the future.


**Figure 2.** Composting larvae species *Hermetia illucens* a), and its frass byproduct b).

**BSFL nutrient content and yields.** The literature surrounding BSFL as a waste management tool has been growing, however, the frass byproduct has only recently generated attention as a possible biofertilizer. This has resulted in sparse and often conflicting reports regarding the nutrient contents and yield potentials. Early indications suggest that BSFL frass tends to have high nutrient contents for an organic fertilizer and a relatively low C/N ratio. Yaacobi et al. (2019) documented frass substrate fertilizer outperforming commercial vermicompost yields on cucumber plants. More research is needed to determine the extent of nutrient and yield contributions.

**BSFL microbial activity.** BSFL possess microflora, which have been attributed with the ability to reduce the concentration of harmful pathogens and chemicals during the digestion process (Lalander et al., 2016). This could enable organic materials that traditionally possess large pathogen populations, to be safely composted. Additionally, the BSFL chitin exoskeleton (a nitrogen containing polysaccharide) is shed and mixed into their frass during the larval growth (Klammsteiner et al., 2020). This results in beneficial chitinolytic microbe activity, which help breakdown chitinous compounds within the soil (Sharp, 2013). Furthermore, adding chitinous material to a soil can also act as an effective pest and pathogen resistant (Hadwiger, 2013).

## Materials and Methods

### Experimental Setup

Two separate composting systems were set up using each particular biological organism to digest the organic material (RW and BSFL). The compost from each system was then analyzed for nutrient content and independently used to determine their effectiveness as a jalapeno seedling substrate. Application efficacy was determined via measurements of stem length, stem

thickness, number of leaves and chlorophyll content via soil plant analysis development (SPAD) readings. A second identical jalapeno experiment was run simultaneously using sterile versions of the substrates to determine microbial relevance to seedling growth. The compost application experiments were replicated three times.

## Composting Systems and Analysis

**compost analysis.** Total elemental P, total elemental K and TKN in all samples were determined by The University of Florida's soil analysis lab.

## Compost Application Experiment and Analysis

**compost application treatments.** The six treatments were as follows:

T1: 4x 4" pots containing a 100% desalinated coco coir substrate
T2: 4x 4" pots containing a 1:1 (mass:mass) ratio of desalinated coco coir/BSFL compost
T3: 4x 4" pots containing a 1:1 (mass:mass) ratio of desalinated coco coir/RW compost
T4: 4x 4" pots containing 100% sterile desalinated coco coir substrate
T5: 4x 4" pots containing a 1:1 (mass:mass) ratio of sterile desalinated coco coir/BSFL compost
T6: 4x 4" pots containing a 1:1 (mass:mass) ratio of sterile desalinated coco coir/RW compost

**substrate sterilization.** T4, T5 and T6 were sterilized prior to seedling transplant by placing 400 g of each substrate in an autoclave for a 90-minute gravity cycle at 121°C.

**data collection.** After three weeks stem length, stem width, number of leaves and SPAD data was collected from each experimental unit.

The plant's SPAD value was determined by taking three readings on the apical most eastwardly facing leaf of the plant and averaging those values.

**data analysis.** Data table analysis for SPAD values, number of leaves, plant height and plant stem width were achieved by calculating the mean and standard deviation (SD) for each treatment using R-Studio.

Treatments were categorized and separated into sterilized and unsterilized data sheets. A Tukey's honest significant difference (HSD) was then run after an analysis of variance (ANOVA) on the three sterilized treatments and each measured variable. The same was done on the three unsterilized treatments and each measured variable. Treatment significance was then determined for each group if $P<0.05$.

Lastly treatments were re-categorized and separated into control, RW, and BSFL data sheets. The significance of sterilization on each measured variable was then determined by running an ANOVA test within each of the three new data sheets. Significance was determined if $P<0.05$.

Outliers for all treatments were designated as a value below Q1($25^{th}$ percentile) -1.5 * IQR (interquartile range) or above Q3($75^{th}$ percentile) + 1.5 * IQR (interquartile range).

## RESULTS

### Processed Waste

After the four-month feeding period, the cumulative mass of food scraps processed in the BSFL composting system was 19.659 kg. The cumulative mass of food scraps processed in the RW composting system over the same period was 14.117 kg (-5.542 kg) (Fig. 3).

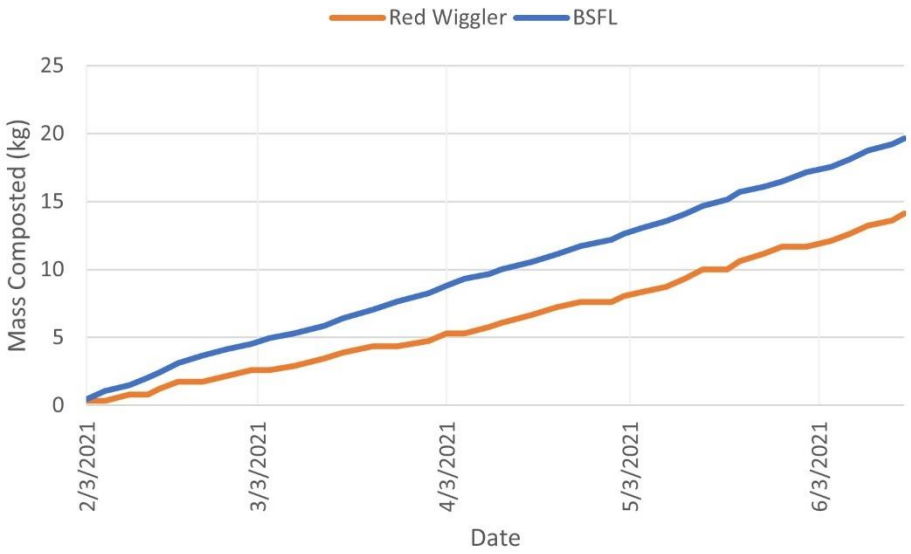


**Figure 3.** Mass of food waste composted (kg), by BSFL (in blue) and RW (in orange), over the course of 4 months

**Nutrient Analysis of BSFL and RW compost**

**pH values.** The BSFL and RW systems appeared to impact the pH levels of the compost produced. The BSFL system produced a pH level of 7.5 after the first analysis and 7.0 after the second analysis. Alternatively, the RW compost displayed a pH level of 6.2 on both analysis dates (March 21 and July 6). On both analysis dates the BSFL system produced a more alkaline compost (+1.3 pH and +0.8 pH respectively) (Fig. 4a).

**TKN concentration.** TKN was determined in both compost systems after 46 and 123 days. TKN concentration in the BSFL compost was measured to be greater than the RW compost on both analysis dates, by +109.4 kg/ha and +165.3 kg/ha respectively

Between the two analysis dates TKN concentration increased within the BSFL compost by +367.3 kg/ha, while increasing within the RW compost by +311.4 kg/ha over the same timespan (Fig. 4b).

**elemental K concentration.** Elemental K concentration was determined in both compost systems after 46 and 123 days. Elemental K concentration in the BSFL compost was measured to be greater than the RW compost on both analysis dates, by +256.5 kg/ha and +721.9 kg/ha respectively.

Between the two analysis dates elemental K concentration increased within the BSFL compost by +672.9 kg/ha, while it increased within the RW compost by +207.5 kg/ha over the same timespan (Fig. 4c).

**elemental P concentration.** Elemental P concentration was determined in both compost systems after 46 and 123 days. Elemental P concentration in the BSFL compost was measured to be greater than the RW compost on both analysis dates, by +30.0 kg/ha and +76.1 kg/ha respectively.

Between the two analysis dates elemental P concentration increased within the BSFL compost by +76.1 kg/ha, while it increased within the RW compost by +30.0 kg/ha over the same timespan (Fig. 4d).

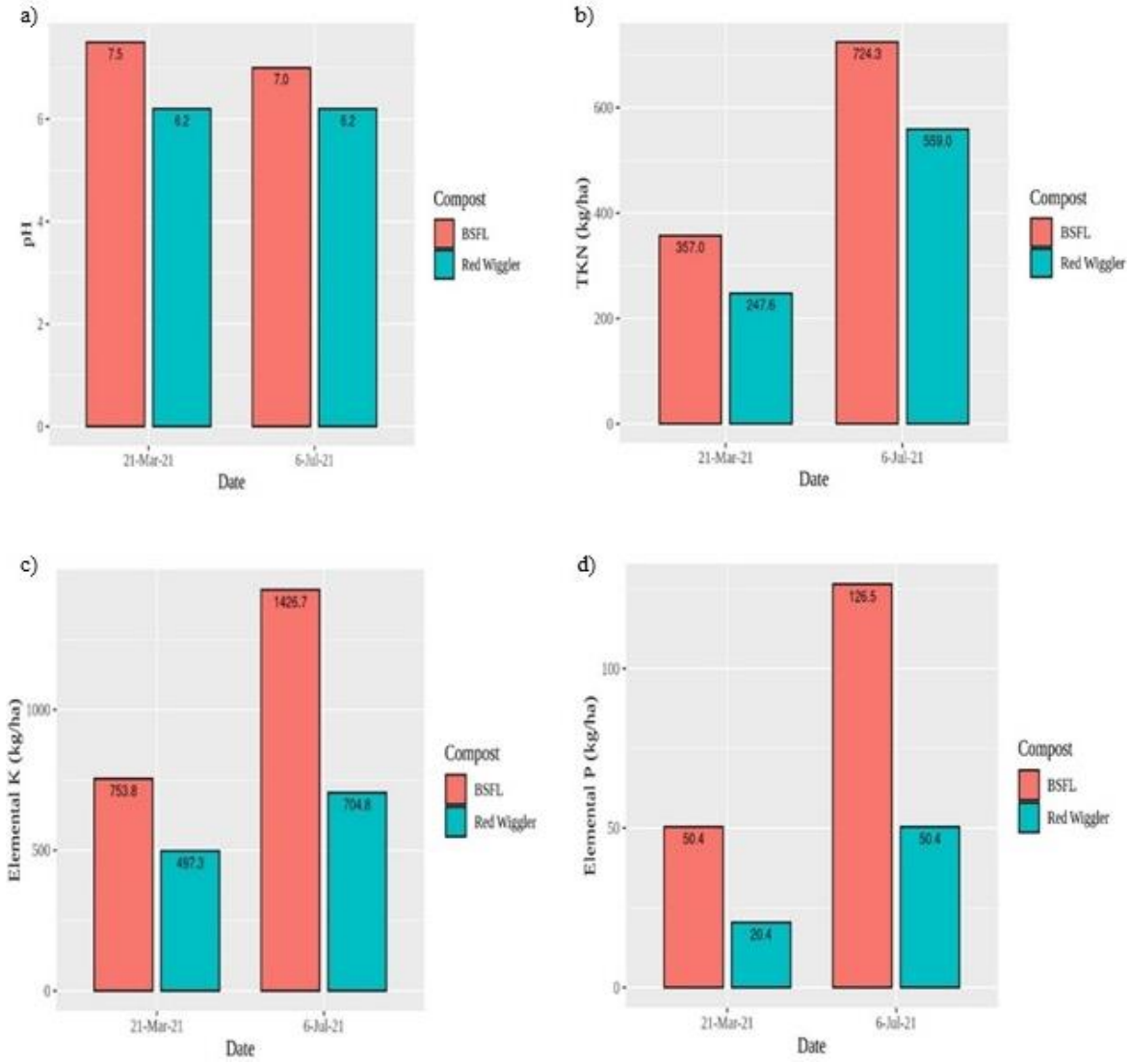


**Figure 4.** Lab analysis after 49 and 110 days for pH value a), TKN concentration b), elemental K concentration c), and elemental P concentration d).

## Application Trials of Compost Substrate from BSFL, RW and Control Treatments

**Table 1.** Mean/standard deviation of SPAD, plant height, number of plant leaves, and stem width, after three weeks

| | SPAD Value ($\bar{x} \pm sd$) | Plant Height (cm) ($\bar{x} \pm sd$) | Number of leaves ($\bar{x} \pm sd$) | Stem Width (mm) ($\bar{x} \pm sd$) |
|---|---|---|---|---|
| **Unsterilized** | | | | |
| Control | N/A | $4.1 \pm 0.8$ | $3.3 \pm 0.6$ | $0.96 \pm 0.2$ |
| BSFL | $17.3 \pm 1.8$ | $6.6 \pm 0.5^{*}$ | $7.3 \pm 0.7^{*}$ | $1.51 \pm 0.1^{*}$ |
| RW | $27.1 \pm 1.7^{\ddagger\,b}$ | $8.3 \pm 0.6^{*\dagger}$ | $11.1 \pm 0.6^{*\ddagger a}$ | $2.91 \pm 0.1^{*\ddagger\,a}$ |
| **Sterilized** | | | | |
| Control | N/A | $3.8 \pm 0.9$ | $3.3 \pm 0.9$ | $0.91 \pm 0.1$ |
| BSFL | $18.3 \pm 1.8$ | $7.0 \pm 0.4^{*}$ | $7.3 \pm 0.3^{*}$ | $1.66 \pm 0.2^{*}$ |
| RW | $23.1 \pm 1.2^{\ddagger}$ | $8.2 \pm 0.6^{*\dagger}$ | $9.2 \pm 1.4^{*\dagger}$ | $2.58 \pm 0.1^{*\ddagger}$ |

N/A = SPAD readings from control treatment were unable to be collected, due to plant size.
* = significant difference from the control (Tukey's HSD after ANOVA test). * P<0.001.
† & ‡ = significant difference from the non-control treatment (Tukey's HSD after ANOVA test). † P<0.05, ‡ P<0.001.
a & b = significant difference from the treatment's sterilized counterpart (ANOVA test). a P<0.05, b P<0.001.

**unsterilized SPAD values.** After three weeks, the unsterilized RW treatment displayed the largest mean SPAD value. Outliers were detected at 31.5 and 23.3 in the RW and BSFL treatments respectively (Fig. 5). The unsterilized RW treatment was shown to have significantly higher SPAD than the unsterilized BSFL treatment. Additionally, the unsterilized RW treatment was determined to have significantly higher SPAD than its sterilized counterpart (Table 1).

**sterilized SPAD values.** After three weeks, the sterilized RW treatment displayed the largest mean SPAD value. An outlier was detected at 7.8 in the BSFL treatment (Fig. 5). The sterilized RW treatment was shown to have significantly higher SPAD than the sterilized BSFL treatment (Table 1).

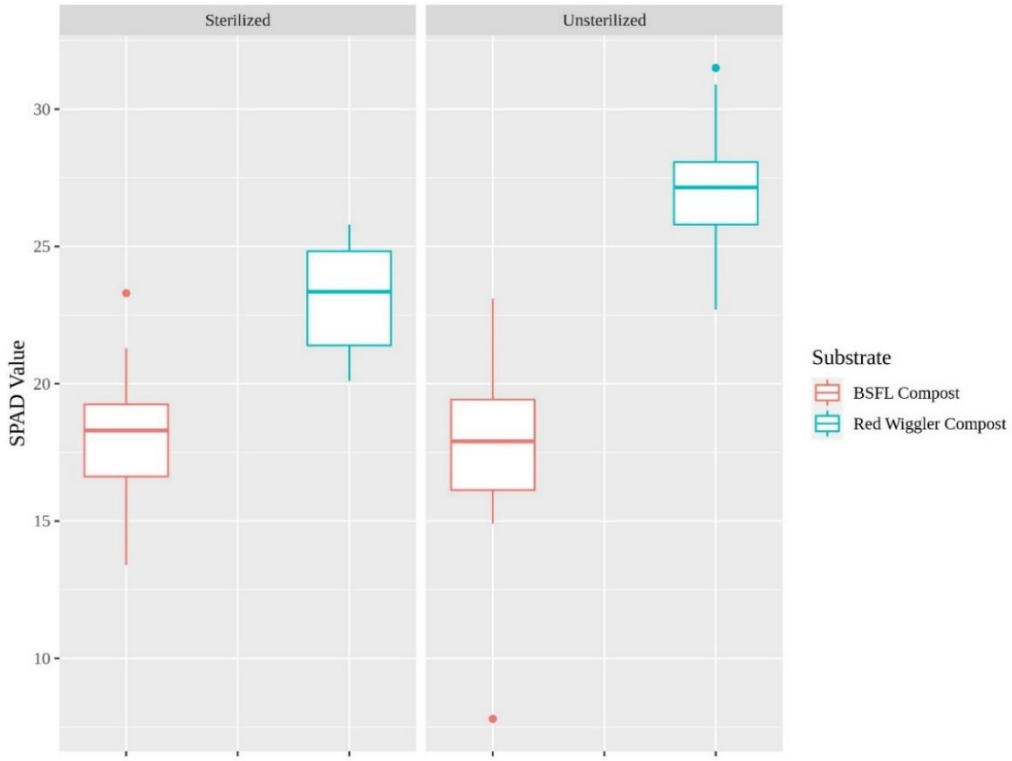


**Figure 5.** Impact of unsterilized and sterilized substrate treatments on leaf chlorophyll via SPAD readings

**unsterilized plant height.** After three weeks, the unsterilized RW treatment displayed the tallest mean height. Outliers were detected at 4.2 cm and 4.3 cm in the RW and BSFL treatments respectively (Fig. 6). The unsterilized RW treatment was shown to have significantly taller plant heights than the unsterilized BSFL and control treatments. Additionally, the unsterilized BSFL treatment was shown to have significantly taller plant heights than the control treatment. None of

the unsterilized treatments were determined to have significantly taller plants than their sterilized counterparts (Table 1).

**sterilized plant height.** After three weeks, the sterilized RW treatment displayed the tallest mean height. Outliers were detected at 5.9 cm and 7.9 cm in the BSFL treatment (Fig. 6). The sterilized RW treatment was shown to have significantly taller plant heights than the sterilized BSFL and control treatments. Additionally, the sterilized BSFL treatment was shown to have significantly taller plant heights than the control treatment (Table 1).

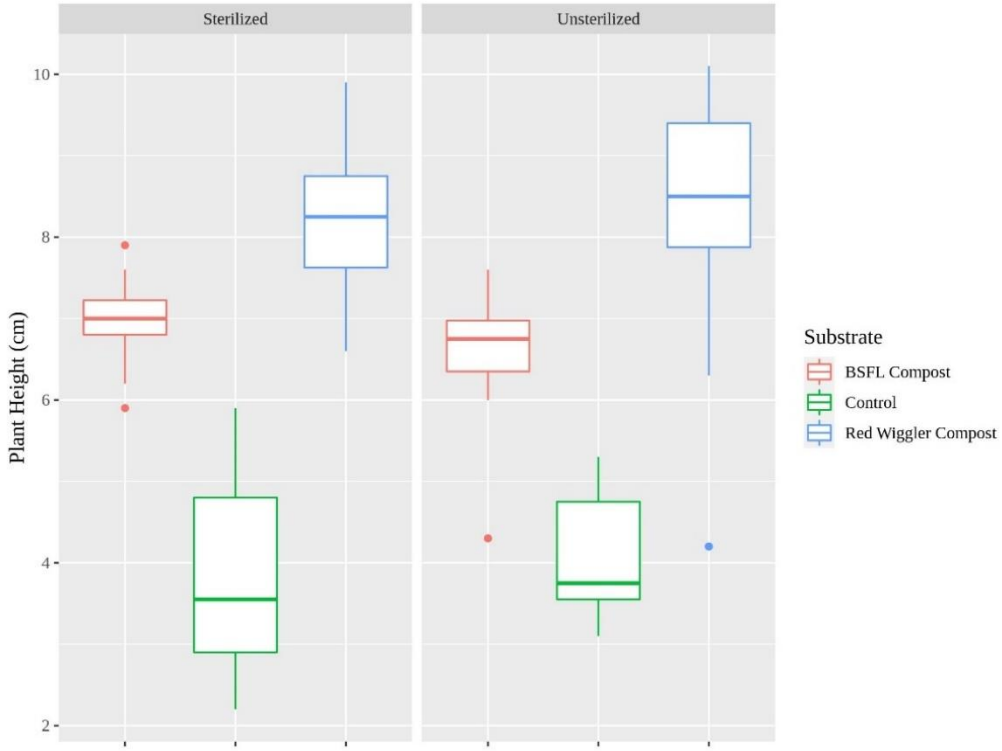


**Figure 6.** Impact of unsterilized and sterilized substrate treatments on plant height readings

**unsterilized number of plant leaves.** After three weeks, the unsterilized RW treatment displayed the most plant leaves. No outliers were detected in any of the treatments (Fig. 7). The unsterilized RW treatment was shown to have significantly more plant leaves than the unsterilized BSFL and control treatments. Additionally, the unsterilized BSFL treatment was shown to have significantly more plant leaves than the control treatment. Lastly, the unsterilized RW treatment was determined to have significantly more plant leaves than its sterilized counterpart (Table 1).

**sterilized number of plant leaves.** After three weeks, the sterilized RW treatment displayed the most plant leaves. No outliers were detected in any of the treatments (Fig. 7). The sterilized RW treatment was shown to have significantly more plant leaves than the sterilized BSFL and control treatments. Additionally, the unsterilized BSFL treatment was shown to have significantly more plant leaves than the control treatment (Table 1).

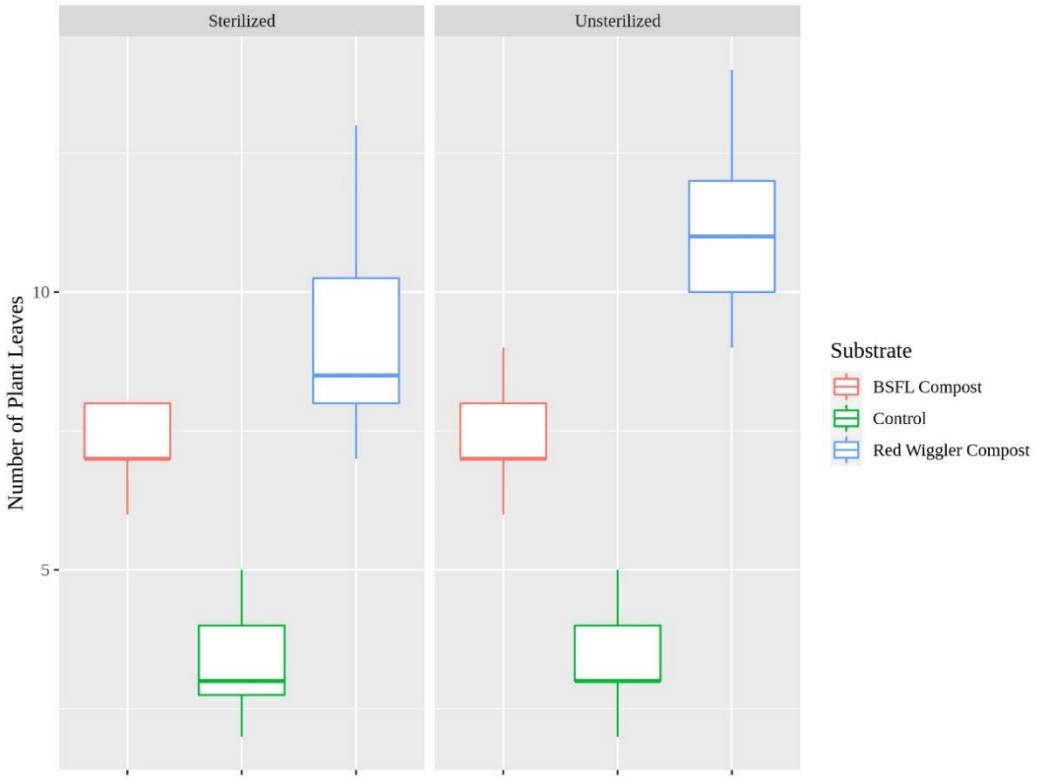


**Figure 7.** Impact of unsterilized and sterilized substrate treatments on the numbner of plant leaves

**unsterilized plant stem width.** After three weeks, the unsterilized RW treatment displayed the thickest mean stem width. Outliers were detected in both the RW treatment (3.64 mm), and the BSFL treatment (1.20 mm, 1.65 mm, 1.90 mm) (Fig. 8).The unsterilized RW treatment was shown to have significantly thicker stems than the unsterilized BSFL and control treatments. Additionally, the unsterilized BSFL treatment was shown to have significantly thicker stems than the control treatment. Lastly, the unsterilized RW treatment was determined to have significantly thicker stems than its sterilized counterpart (Table 1).

**sterilized plant stem width.** After three weeks, the sterilized RW treatment displayed the thickest mean stem width. No outliers were detected in any of the treatments (Fig. 8). The

sterilized RW treatment was shown to have significantly thicker stems than the sterilized BSFL and control treatments. Additionally, the unsterilized BSFL treatment was shown to have significantly thicker stems than the control treatment (Table 1).

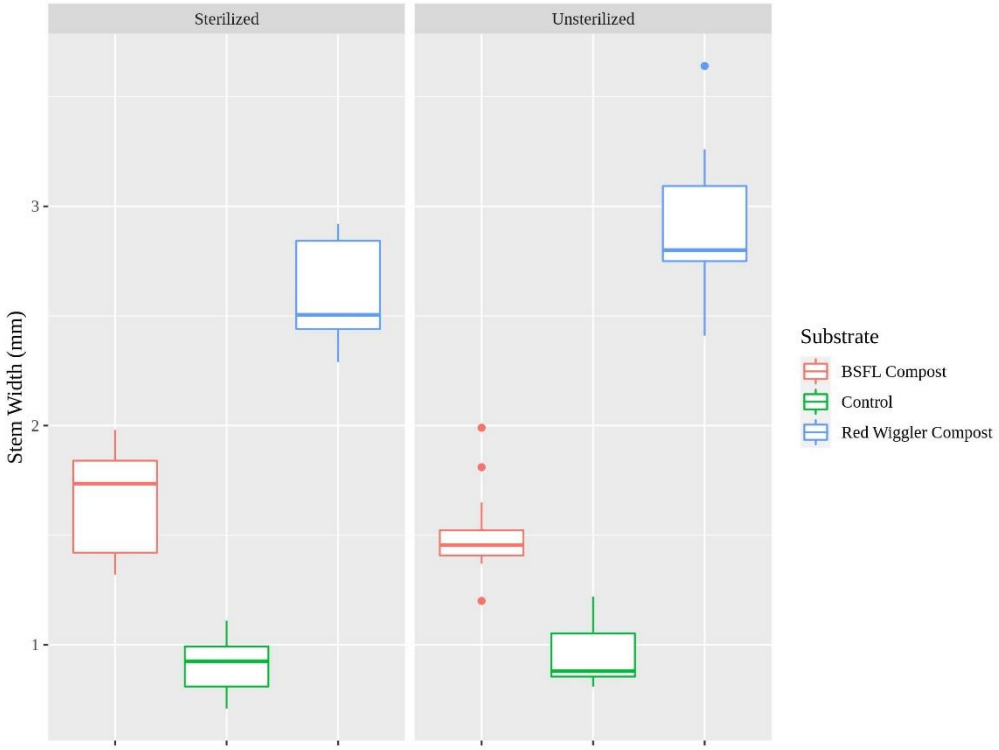


**Figure 8.** Impact of unsterilized and sterilized substrate treatments on plant stem width

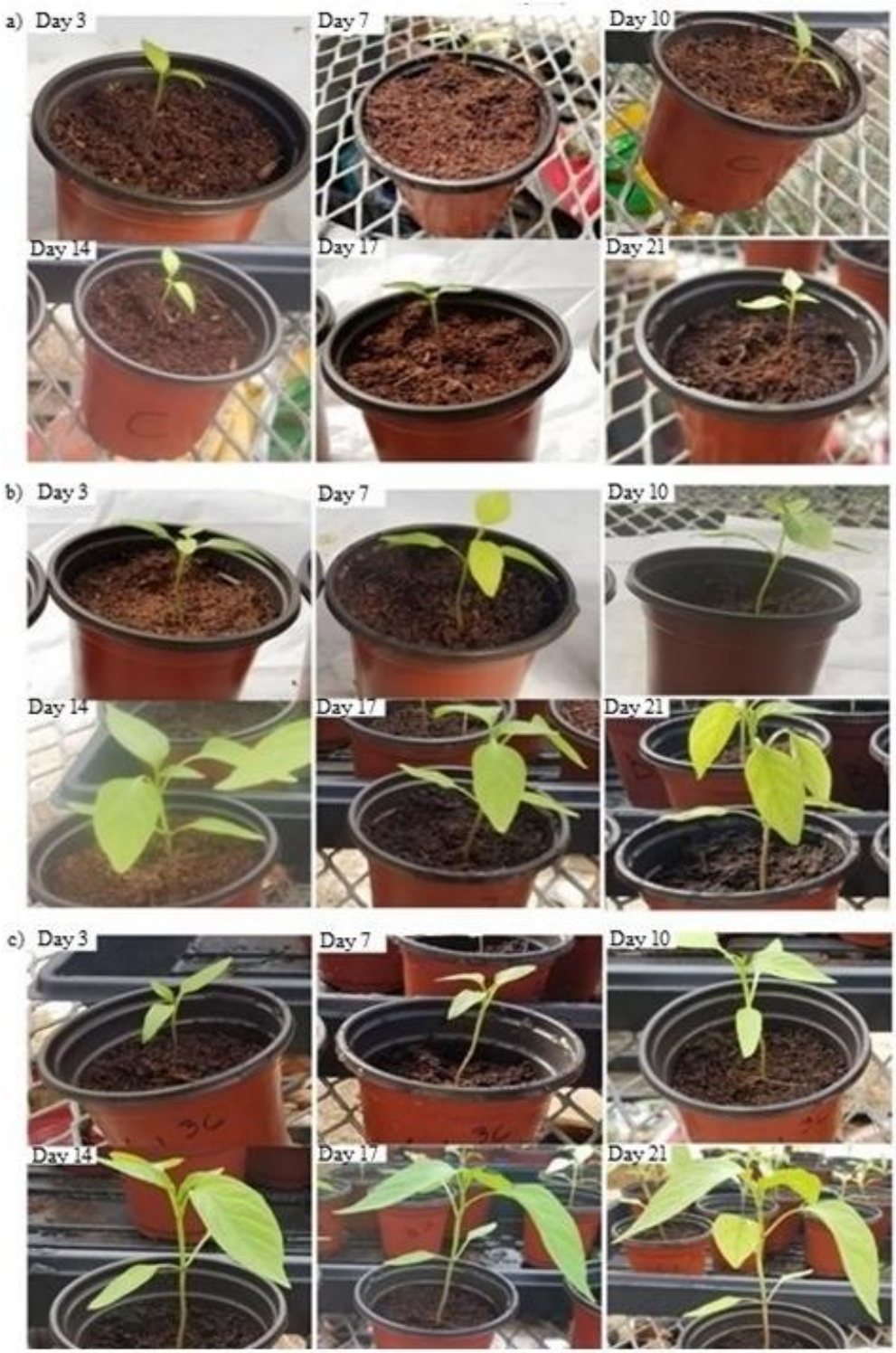


**Figure 9.** Impact on growth progress of *Capsicum annum* by unsterilized treatments T1 a), T2 b), and T3 c).


**Figure 10.** Impact on growth progress of *Capsicum annum* by unsterilized treatments T4 a), T5 b), and T6 c).

## DISCUSSION

### Waste Consumption

Clearly, the waste consumption rate of BSFL was greater than that of RW worms. By considering the life cycles of each organism, we can better understand the variability in this waste consumption. While RW undergo a period of rapid growth leading up to their reproductive

stage, they have been shown to exhibit a growth regression stage upon reaching sexual maturity (Ali & Kasheem, 2018). In comparison, BSFL larva will likely spend their entire larval stage consuming as much sustenance as they are able, thus accumulating the necessary energy stores for metamorphosis and subsequent non-consuming stages (Arrese & Soulages, 2010). Kim et al. (2011), underscores this by reporting a much higher activity from a large array of gut enzymes in BSFL when compared to other common fly larval species.

**Nutrient Content**

BSFL contained noticably higher N, P and K concentrations. By considering the extremely active digestive enzymes of BSFL, we can assume more macromolecules will be present in this system, due to the larger quantities of waste processed. Additionally, protease and trypsin-like protease enzyme classes are primary sources of catalyzing the breakdown of protein/peptide molecules. Due to the highly active nature of these enzymes in BSFL digestion it is plausible that they account for some of the elevated N and P in the BSFL compost (Kim et al., 2011). Furthermore, BSFL are known to consistently shed their chitinous exoskeletons as they progress through several development stages. These N-rich chitin molecules could noticeably contribute N to a BSFL compost (Tharanathan & Kittur, 2003; Klammsteiner et al., 2020).

**Application Trials**

RW compost significantly overperformed across all four growth measurables. These outcomes clearly outline the fact that vigorous seedling growth is more than simply administering a more abundant nutrient source. Compost immaturity may have also hampered BSFL growth outcomes. There is some evidence that suggests further forced aeration techniques may be needed to fully break down toxic phenol phytotoxins within the compost's chemical structure (Song et al., 2021). By applying compost in an immature state, the plant may be subjected to toxic levels of these phytotoxins during its vulnerable seedling stage. Furthermore, immature compost continues to be decomposed at a high rate after application, this respiratory process can reduce available oxygen and nutrients around the root zone, as they are utilized by microbes (Brinton, 2000).

**Microbial Sterilization**

Sterilization of the BSFL compost resulted in no significant correlation among the four recorded growth measurables. This could suggest that although BSFL compost microbes have shown the ability to minimize growth reducing pressures like pest/pathogen, they may not significantly contribute to growth promoting factors. On the other hand, the RW compost displayed a significant correlation between substrate sterilization and three of the four growth measurables (SPAD, stem width, and number of leaves). With these results we can assume that a percentage of the plant growth, upon the application of vermicompost, can be attributed to the beneficial microorganisms present.

## CONCLUSION

This experimental work is a start in determining the effectiveness of using BSFL in organic waste management and the potential of its frass byproduct. During the research, the potential for BSFL to quickly digest waste into a value-added product was clearly shown. Additionally, BSFL frass has a comparatively superior concentration of N, P, K nutrients, over RW castings. Lastly, BSFL frass was shown to be a beneficial organic input compared to no input at all. All of this suggests that it could be a highly beneficial biofertilizer input. However, it was found to be comparatively less effective than RW compost. These inferior results could be due to the excellent physical characteristics of RW compost, a need for additional maturing processes in BSFL compost, and to a lesser extent, more growth promoting microbes in RW compost. Future research could be done comparing RW compost to BSFL compost which has undergone additional maturing processes, to determine whether BSFL application results can be made more comparable to RW application results.

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