Mealworm frass and vermicompost tea as novel plant-ready nutrients for indoor hydroponics bok choy production system

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2 authors, including:

Lillian Wang
Bennett Aerospace

MEALWORM FRASS AND VERMICOMPOST TEA AS NOVEL PLANT-READY NUTRIENTS FOR INDOOR HYDROPONICS BOK CHOY PRODUCTION SYSTEM

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THESIS: MEALWORM FRASS AND VERMICOMPOST TEA AS NOVEL PLANT READY NUTRIENTS FOR INDOOR HYDROPONICS BOK CHOY PRODUCTION SYSTEM

AUTHOR: Lillian F. Wang

DATE SUBMITTED: Fall 2022

Department of Plant Science

Dr. Eileen Cullen
Thesis Committee Chair
Plant Science Department Chair ____________________________________

Dr. Valerie Mellano
Professor of Plant Science ____________________________________

Dr. Aaron Fox
Associate Professor
Of Plant Science ____________________________________

Duncan McKee
Soil Lab Technician and Instructor
Of Plant Science ____________________________________

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ABSTRACT

Nutrient film technique (NFT) is a type of soil-less hydroponic production system that is water efficient with high yield outputs. NFT systems currently rely on synthetic inorganic chemical nutrients fully dissolved in water that provide macro-, micro- and trace- mineral nutrients for plant growth. The synthetic source of conventional hydroponic nutrients lowers NFT sustainability. *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), or mealworm larvae, is commonly reared for animal feed and produces frass - insect waste. Vermicompost, a nutrient and microbe-rich medium of decomposed organic matter produced by earthworms, has been researched extensively on their positive effects on plant growth and plant immunity. The research on the performance of frass is severely limited in agriculture; vermicompost studies in hydroponics have had varying success. To curb the reliance of hydroponic systems on synthetic inorganic mineral nutrients, this thesis investigates the potential of *T. molitor* frass and vermicompost as standalone hydroponic fertilizers. *Brassica rapa* ssp. *chinensis*, commonly known as bok choy, were grown in three NFT systems, each system with one of three treatments: frass tea, vermicompost tea, and the control, which was a commercial synthetic inorganic mineral nutrient hydroponic fertilizer. The control outperformed the two organic hydroponic fertilizer treatments in terms of appearance, which was measured in the form of chlorophyll concentration, and yield. Through 95% confidence intervals (CI), frass, vermicompost, and the control were statistically different from one another. The control outperformed both frass and vermicompost treatments in terms of chlorophyll concentration and fresh harvest weight. Frass had the second highest level of chlorophyll concentration, and vermicompost had the second highest fresh harvest weight. Results demonstrated the potential of integrating frass waste into hydroponic food production systems and could encourage sustainable food production practices by decreasing the amount of synthetic fertilizer required in hydroponic systems.

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INTRODUCTION

Hydroponics is a form of soil-less farming that efficiently uses water and dissolved mineral nutrients for plant growth compared to traditional field agriculture. Hydroponic systems are typically indoors, thus they are especially popular among leafy green and herb growers where they can better manage pests and adjust the growth climate (George and George, 2016). Sustainable agriculture includes not only food production, but also rearing insects such as the black soldier fly (*Hermetia illucens* L.; Diptera: Stratiomyidae) and mealworms for animal feed. Yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is commonly used as a feed source for poultry, aquaculture, and pets (Grau et al., 2017; Biasato et al., 2016). The rearing process requires minimal resources and its waste by-product, frass, has the potential to be a plant-ready nutrient source that can be readily used in hydroponics (Houben et al., 2020; Barragán-Fonseca et al., 2022, Bloukounon-Goubalan et al., 2021). This project examined the potential of *T. molitor* frass as a viable organic nutrient source for hydroponic fertilizer by comparing its effectiveness to vermicompost extract and commercial synthetic inorganic mineral nutrient solution.

Hydroponics

The continuous population growth translates to an increase in global demand for food (Godfray et al., 2010). With the increased global demand for food comes with increasing the stress on finite resources including water. The concern over global water scarcity that has paved way for measures to alleviate water scarcity and improve sustainability (Kummu et al., 2016). Improving sustainability in agriculture includes efficient water-use (Pretty, 2008; Forouzani and Karami, 2011). Investing in improved irrigation technology to increase irrigation efficiency can benefit farmers. They may feel incentivized with increased income, convenience, reduced costs, and minimize yield loss risks (Perry et al., 2009). On top of improved irrigation technology, there has been an increased interest in water-efficient growing systems that deviates from traditional field agriculture, one example of that would be hydroponic systems.

The term “Hydroponics” was coined and popularized by Dr. W. F. Gericke, a University of California researcher back in the 1930s when he wrote his book *The complete guide to soilless gardening* (Gericke, 1940). This soilless growing method relies on mineral macro- micro- and trace nutrient solutions to support the growth of plants. Roots may be physically supported by inert mediums such as gravel, perlite, and rock wool, but it can also forgo mediums with the roots submerged in the nutrient solution only. In terms of water use efficiency, a closed hydroponic nutrient solution system can save up to 90% of irrigation water compared to traditional agriculture (Alshrouf, 2017). Hydroponic systems not only conserve water but are also highly productive. One study in Arizona, USA compared lettuce production yield between using nutrient film technique (NFT) and traditional agriculture, and the NFT system produced 11 times higher yields compared to traditional agriculture (Barbosa et al., 2015). However, the same Arizona study determined that the energy requirements for hydroponically produced lettuce were 82 times higher than conventionally produced lettuce and identified energy availability as a major factor when considering the sustainability of hydroponic food production systems (Barbosa et al., 2015). Renewable energy such as solar, geothermal, or wind power, can be used to offset the energy requirements of hydroponic food production. This can be an attractive method of food production for regions that have abundant renewable sources of energy. There are two main classifications of hydroponics systems : active systems and passive systems (George and George, 2016). Passive systems rely on the plant roots directly in contact or submerged in the nutrient solution. It is a basic system that is portable and can be inexpensive but may encounter issues with supporting larger, heavier plants (George and George, 2016). The following factors should be considered before selecting a technique: space, expected productivity, growing medium availability, and expected produce quality (Sardare and Admane, 2019). One example of a passive system is the wick system, where a lamp or nylon wick is used to supply nutrient solution to the plant root, using vermiculite, perlite, or LECA as the growth material (George and George, 2016). On the other hand, active systems are more efficient and productive due to its reliance on pumps supplying nutrient solutions directly to the plants and drain excess solution that can be reused and recycled using gravity. Active systems rely on the water pumps integrated into the system to maintain the plants. If the pumps fail, the plants run the risk of desiccation (Sardare and Admane, 2019). A few examples of active hydroponics systems include the ebb and flow system, raft method, and NFT. Ebb and flow is also known as a flood and drain system. Ebb and flow requires a lower cost to set up and maintain and can be automated with a computerized timer attached to the pump. The raft method requires stiff Styrofoam sheets to support plant growth as it keeps the plant root submerged in the nutrient solution and the rest of the plant afloat. The pumps underneath the Styrofoam sheets will promote circulation and reduce solution stagnation (George and George, 2016). The NFT system is the hydroponic system chosen for this thesis project.

Nutrient Film Technique (NFT)

The concept of NFT was developed by Dr. Allen Cooper in 1965 (Burrage, 1993). It is a modular hydroponic technique where a shallow film-like stream of mineral nutrient-rich water flows and recirculates through the bare roots of plants in sloped channels. It maintains a coating of nutrient solution around the roots, without the use of a physical root support substrate (van Os et al., 2019). Plant growth requires proper air exchange in the root systems, which can be achieved by having just a few millimeters depth of nutrient solution flowing through the channels constantly, hence the name nutrient "film" technique. *Nutrient film technique - crop culture in flowing nutrient solution* by Spensely et al. (1978) details the setup and equipment required for NFT systems and provided a general framework that is still applicable today. The solution flows through the system via a combination of a pump pushing water from the sump into the channels (often referenced as "gullies" by Spensely et al.), and gravity pulling the water through the sloped channels (Figure 1.). Channels can be formed from polythene, which are affordable with no stabilizers or additives that can seep into the solution and cause phytotoxicity (Spensely et al., 1978). NFT is a productive system that relies heavily on a stable source of energy to power pumps that transport nutrient solutions through the system. Therefore, the system risks having little protection against flow interruption if there is a power supply disruption and having "fail-safe" equipment such as generators is recommended (Spensely et al., 1978). However, there have been technological advancements that can subvert the risks of flow interruption such as a combination of agricultural sensors (Peuchpanngarm et al., 2016) and automated power supply (Sihombing et al., 2018). The flowing nutrient solutions in NFT systems can potentially allow plants to have a more flexible access to a larger volume of nutrients NFT is a closed system that is vulnerable to poor water quality and the spread of disease throughout the entire system, and diligent testing of the water quality for diseases and ion imbalances is crucial for successfully operating NFT systems.

Hydroponic Nutrient Solutions

There are a few key elements that are universally accepted to be core factors to functioning hydroponic nutrient solution: mineral composition, pH, and electrical conductivity (EC) (de Rijck and Schrevens, 1995; Sambo et al., 2019). Jones (1982) discussed the history of hydroponics and its use in plant nutrition studies, which later became the basis of an early recipe for hydroponic nutrient solutions. Scientific interest in what promotes the growth of plants budded during the 1700s when scientists experimented with different mixtures of soil and water, but it was not until the 1800s when scientists started determining what mineral nutrients were crucial for plant growth and development. Wilhelm Knop, an agrochemist, developed a standard nutrient solution that is still commonly used today (See Table 1 for Knop's Nutrient Solution) (Jones, 1982). Knop's standard solution emphasized balancing the major inorganic nutrients with considerations to its osmotic pressure and avoiding precipitation forming during its use as well as the proportion of the nutrients relative to one another. Knop's standard solution was experimented with and modified, which later became a widely accepted nutrient solution formula. The nutrient solution formula was the Arnon and Hoagland nutrient solution, more colloquially known as just Hoagland's nutrient solution (See Table 2 for Hoagland's nutrient solution) (Jones, 1982). Many different nutrient solutions would later be developed and used depending on crop need. However, by the 1860s Knop had already established the essential elements needed for plant growth, which are nitrogen, phosphorus, potassium, magnesium, calcium, iron, and sulfur (Jones, 1982).

In addition to mineral composition, pH is crucial to plant growth and development. Steiner (1961) noted that to formulate the optimum nutrient solution for any crop requires a systemic investigation to the relative ratios of the nutrient ions, the total ionic concentration, and the pH. The pH is used to indicate the relationship between concentration of H^+ and OH^- free ions, meaning the acidity or alkalinity of a solution. Nutrient solutions must contain plant absorbable chemical ions, and plant productivity is correlated with proper nutrient uptake governed by pH regulation (Trejo-Téllez and Gómez-Merino, 2012). The pH of nutrient solutions affects dissociation, complexation, and precipitation reactions that could impact speciation and bioavailability in hydropic crop production (De Rijck and Schrevens, 1997). The ions that determine the pH of nutrient solutions are dihydrogen phosphate ($H_2PO_4^-$), bicarbonate (HCO_3^-), and ammonium (NH_4^+) (De Rijck and Schrevens, 1997). Commercially, nutrient solutions contain all three, with the pH determined mainly by the relative concentrations of $H_2PO_4^-$ and HCO_3^-. McCaulley et al. (2009) also noted the effects of pH on plant-available nutrients hydroponic nutrient solutions. Phosphorus (P) is the most available within a 6 to 7 pH range, and macronutrients such as nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are more available when the pH range is between 6.5 and 8 (McCaulley et al., 2009). The majority of the micronutrients such as iron (Fe), zinc (Zn), and nickel (Ni) would be more available between the pH range of 5 and 7 (McCaulley et al., 2009). This indicates that pH is crucial for nutrient management in a hydroponic system and should be closely monitored and frequently measured.

Another essential component of a viable nutrient solution is the electrical conductivity (EC). EC is the potential of any material to conduct electricity. In a hydroponics setting, the EC is used to detect electricity conducted by ions from dissolved salts. The concentration of free-floating ions from dissolved salts in nutrient solutions indirectly indicates the balance of plant available nutrients in a nutrient solution (Samarakoon et al., 2006). Ions associated with salinity are Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, H^{+}, $NO3^{-}$, $SO4^{-}$, Cl^{-}, $HCO3^{-}$, and OH^{-} (United States Department of Agriculture [USDA], 2001). Salts are essential for plant growth. However, in excess, these ions in salt form would affect plant growth by direct toxicities. This disrupts the ionic balance of the plant, interferes with nutrient uptake, lowers water availability by compromising the osmotic potential. In fields, high sodium ions can deteriorate soil structure by dispersing soil clays (USDA, 2001). An important factor to consider while measuring EC is the temperature of the nutrient solution, as the EC of a solution was shown to increase 1.9% per 1-degree Celsius increase (American Public Health Association, 2005). To prevent over or underestimation of EC values, nutrient solution temperatures need to be noted before EC levels are measured.

Frass and Vermicompost as Plant Nutrient Sources

Across the globe, people have farmed and reared insects for varying reasons. Some rear insects for biological control in agriculture (Singh, 2012), for research, and as food source for animals and humans (Looy et al., 2014; van Huis, 2013; Bessa et al., 2017). Scientists and environmentalists are interested in insect rearing as a potential solution to food insecurity and unsustainable food production (Siemianowska et al., 2013; Grau et al., 2017). However, there are still concerns surrounding the sustainability aspect of insect rearing for human consumption as the edible insect industry does not consider sustainability the main priority (Wade and Hoelle, 2020). Instead, the insect industry prioritizes consumer behavior such as price, taste, and availability (Berger et al., 2018). Regardless, frass will be a by-product of the insect rearing industry, and this research project can propose a solution to repurposing frass to valorize or upcycle waste from mass insect production.

Vermicompost

Vermicompost is the excreta of earthworm, also referred to as worm castings, a nutrient-rich substrate that can improve soil health. This nutrient-rich substrate is produced via earthworms passing biodegradable wastes such as kitchen, farm, market, and agro-based bio-wastes through the worm-gut to produce nutrient rich vermicompost (Adhikary, 2012). Vermicompost has high nutrient levels and abundant microbial life and may enhance soil organic matter (Yatoo et al., 2021). Vermicompost may also be superior to compost due to the advantages of shorter production time (Sánchez-Monedero et al., 2001), less phytotoxicity (Belda et al., 2013), more phytohormones (Ravindran et al., 2016; Joshi et al., 2015), and less pathogens (Contreras-Ramos et al., 2005; Kadam et al., 2008). The fertilizing effects of earthworm casts have been investigated by Joshi et al. (2015) stating that the microbial metabolites of earthworm casts influence plant metabolism, growth, and development. Haghighi et al. (2016) have observed the effects of vermicompost as fertilizer in hydroponically grown tomato (*Lycopersicum esculentum* L.). Haghighi et al. have noted in their experiment that vermicomposted municipal solid waste compost significantly increased the number of red tomato fruits in the harvest period compared to the control due to the presence of plant-growth promoting substances present (Haghighi et al., 2016; Zhang et al., 2014). This led to Haghighi et al. concluding that vermicompost has the properties to improve tomato growth physiology in hydroponic culture. The byproduct of vermicomposting includes vermicompost leachate – a nutrient-rich solution that collects at the bottom of worm bins. There have been research that examined the efficacy of vermicompost leachate as liquid fertilizer for sorghum (Gutiérrez-Miceli et al., 2008) and sugarcane (Gutiérrez-Miceli et al., 2017). Gutiérrez-Miceli has concluded that vermicompost leachate has stimulated plant development for both sorghum (2008) and sugarcane (2017) that in order to push for maximum plant growth, NPK fertilization was required. This research will examine the effects of vermicompost leachate on *Brassica rapa* ssp. *chinensis* growth.

Frass

Similar to vermicompost, frass is insect excrement that has impact on soil fertility from its high nutrient content (Kagata and Ohgushi, 2012). Houben et al. (2020) performed a chemical characterization of mealworm (*Tenebrio molitor L.*) frass and revealed the relative concentrations of N, P, and K are similar to those found in farmyard manure, especially poultry. Houben et al. (2020) has also suggested that mealworm frass may increase microbial metabolic activity and diversity, which can improve soil functioning. Barragán-Fonseca et al. (2022) have also noted that frass has the potential to improve plant growth and even pathogen resistance under soil-grown conditions.

Many companies began selling frass as a new alternative to conventional fertilizer, yet there is limited information on frass and its ability to improve plant growth, and research is urgently needed (Berggren et al., 2019, Poveda et al., 2019). Current research suggests that mealworm frass, based on the feed given to the mealworms, can contain plant growth promoters (Poveda et al., 2019). Poveda et al. (2019) also underlined the associated microbiota that can be found in mealworm frass that can be used as a biofertilizer for organic farming. Currently, there are soil studies that have inspected the effects of frass on plant growth (Kagata and Ohgushi, 2012; Klammsteiner et al., 2020; Houben et al., 2020), and frass composition analysis studies (Beesigamukama et al., 2020), but little research is done in a hydroponic setting. One research that involved mealworm frass in a hydroponics setting was used in combination with animal manure, which the results were compared with inorganic Hoagland nutrient solution and a combination of Hoagland and the organic animal-insect nutrient solution (Desaulniers Brousseau et al., 2022). This project aims to evaluate the potential of mealworm frass becoming an alternative standalone fertilizer option for hydroponic farmers.

Bok Choy

Brassica rapa ssp. *chinensis*, commonly known as bok choy, bok choi, pak choy etc., is a type of nutritionally dense Chinese cabbage that is popular for consumption in East and Southeast Asia. Its popularity in the United States can be attributed to its mild flavor and its suitability as a stir-fry vegetable. There are many cultivars of *B. rapa ssp. chinensis* that are suitable for growth such as Black Summer, Feng Qing, Joy choy, and more. Black Summer was the cultivar chosen for this research project, which is described as having a broad, vase-shaped head, with dark green leaves and light green petioles (Nair and Irish, 2016). *B. rapa ssp. chinensis* is a crop that has a short growth period where it will reach maturity between 40 and 60 days after seeding. On top of having a short growth period, popular cultivars such as the Black Summer, Win-Win choy, and Mei Qing choy are also heat tolerant, which is ideal for growing in the summer at the campus greenhouse.

CHALLENGE STATEMENT

There is a demand for food across the globe that can be partially met through hydroponically grown food. In the United States alone, controlled environment food production wholesale value increased from $64.2 million to $796.7 million (adjusted for inflation) and over half of producers employed strictly hydroponic techniques (Walters et al., 2020). Hydroponics production requires plant-available nutrients, often in the form of synthetic fertilizers, for optimum plant growth. Insect rearing may create a substantial amount of frass (Poveda, 2021), which has the potential to become a complete fertilizer for hydroponics systems. The challenge of this project was to determine if hydroponic tea made from frass or vermicompost leachate are adequate sources of plant-ready nutrients for hydroponically grown *B. rapa* ssp. *Chinensis* in a nutrient film technique (NFT) system by comparing the two with a commercially available synthetic hydroponic fertilizer commonly used in large- scale hydroponic production farms. This research project can potentially demonstrate alternative sources of plant-ready nutrients for hydroponic farmers, increase food production, potentially lower operation costs, and boost agricultural sustainability by integrating insect rearing waste management into hydroponic food production systems.

MATERIALS AND METHODS

Location

The experimental portion of the project was conducted in the Cal Poly Pomona campus AGRIscapes greenhouse in Pomona, California, USA from April 2022 to August 2022 (34°02'52.8"N 117°49'07.0"W).

Bok Choy (*Brassica rapa* ssp. *Chinensis*) Germination and Growth Requirements

Seeds were purchased from Johnny's Selected Seeds (Winslow, ME). The variety used for this experiment is the Black Summer (F1) Green Seed. Seeds were placed in Rootcubes® by Oasis® Grower Solutions with 45 days expected to reach maturity. The seeded Rootcubes® were kept moist with greenhouse tap water until the seeds germinated. The pH requirement of bok choy is between 5.5-6.5 and the EC requirement is between 1.5-2.5 (Yadev, 2020). The pH was adjusted using General Hydroponics pH Up Liquid Base Solution and General Hydroponics pH Down Liquid Acid Solution (www.generalhydroponics.com) after the EC requirements were within range. After 20 days of germination, the germinated seedlings were transferred to the NFT channels along with the Rootcubes® that they were planted in. After 30 days the plants were harvested and measured for chlorophyll and fresh weight above the Rootcubes®.

Nutrient Solution (Frass, Vermicompost, Control)

To prevent any diseases from negatively impacting the plants, the NFT systems had a 10% diluted store-bought vinegar solution added into the sump and was left running through the system overnight. Afterwards, the vinegar solution was drained out of the systems, the sump was rinsed clean and let run overnight with water before a 5%

diluted bleach solution was added to the sump to surface sterilize the system. There was one treatment per NFT system for a total of three treatments. The treatments were mealworm frass extract (FE), vermicompost leachate (VL), and an inorganic nutrient solution as the control (GRO). Frass was sourced from KIS Organics (Chehalis, WA), product name is Mealworm Insect Fertilizer (Frass) with an N-P-K ratio of 2-3-2. The obtained frass in dried powder form with minimal processing can be described with a range of shades from light brown to pale yellow with a mild odor akin to the smell of oats (under the pretense that the mealworms were on an all-oats diet). Vermicompost was sourced from Black Diamond Vermicompost (Paso Robles, CA), product name is Black Diamond Vermicompost. The inorganic control was sourced from GroMore, a 20-20-20 N-P-K hydroponic water-soluble fertilizer, accompanied by Cal- Mag, a calcium-magnesium supplement. The obtained vermicompost was akin to damp soil in terms of smell and texture, with a rich dark brown color, and was not as uniform in consistency compared to the frass. The control hydroponic fertilizer (GroMore 20-20-20) formulation was based on Hoagland's nutrient solution recipe. The greenhouse tap water at the Cal Poly Pomona campus AGRIscapes greenhouse was sampled and submitted for agriculture water testing to Denele Analytical Inc. (Turlock, CA) to establish a baseline prior to the start of the experiment (Table 3) before the frass and vermicompost were added. The parameters tested were pH, EC, soluble salts, nitrate nitrogen, nitrate, bicarbonate, Ca, Mg, Na, K, boron (B), chloride, sulfate, and the sodium absorption ratio. Frass and vermicompost samples were also submitted to Denele Analytical Inc. (Turlock, CA) for nutrient analysis prior to making of FE and VL treatment hydroponic nutrient solutions (Table 4, Table 5). The parameters tested for frass and vermicompost were moisture (H_2O), pH, EC, B, Zn, Fe, Mn, Cu, organic matter, K, total phosphorus, Na, Ca, Mg, S, total nitrogen, Soluble salts, and C:N ratio.

FE and VL Formulation

The procedure for making FE and VL require both treatments to enter the acceptable range of EC and pH for bok choy growth. Despite to the differences in mineral components for frass and vermicompost (see Tables 2 and 3), similar amounts (a total of 3095.37 grams of frass and 3088.61 grams of vermicompost) were added to separate 200-micron mesh bags to make 50 gallons each of FE and VL. The mesh bags were submerged and agitated in water for at least 30 minutes until EC readings were within the optimal range for bok choy growth. Afterwards, all solution agitation and aeration came from the sump pump and the nutrient solution collected and returned through the rain gutters. Each extract solution was then passed through a 100-micron mesh bag to catch any finer debris, then finally added to each NFT system sump. This procedure was repeated twice to make a total of 40 gallons per treatment. The control solution was made according to the manufactured label until the EC was within range. Once all three treatments had their EC within range acceptable for bok choy growth, pH adjustments were made using General Hydroponics pH Up Liquid Base Solution and General Hydroponics pH Down Liquid Acid Solution (www.generalhydroponics.com).

Nutrient Solution Testing for NPK, Diseases, Pests and Sanitation

A pH meter with a built-in EC reader was used daily to measure the hydroponic solution nutrient levels. The nutrient solution fluctuates over the course of the experiment. To minimize external variables that relate to the efficacy of the nutrient solution, there were no additional FE, VL, or GRO solutions that were added to influence the changes in the EC range.

Experimental Layout

There were three NFT systems set up in the campus greenhouse. Eighteen plant receptacles per NFT channel, 7 channels per system, for a total of 126 plants per NFT system (Figure 3). Each system received one of the three treatments. The three systems were placed adjacent to each other with 4 ft. of walking space in between in the order of VL, FE, and control from outermost to innermost of the greenhouse.

Data Collection

There were 105 plants harvested from each NFT system (N=315), of which were all weighed (g) and the chlorophyll levels all measured (µg /cm^2) according to the methods section. Chlorophyll levels were measured prior to harvesting using an atLEAF (Wilmington, DE) electronic hand-held chlorophyll meter adhering to manufacturer instructions. Leaf counting was implemented where chlorophyll was sampled from the fourth oldest leaf (excluding the cotyledons). If the fourth oldest leaf was unavailable, chlorophyll was measured from the next best leaf unless there are no leaves of adequate size available on the entire plant. Each head of bok choy was then harvested above the roots and the Rootcubes®, along with the roots, were discarded. Fresh weight was collected by weighing each plant immediately after harvest, recorded in grams using a standard electronic scale.

Statistical Analysis

One-sample statistical inference by:

$$\mu \pm [(t.05);\ df = N-1] \times (S_{\text{x}} \text{ of mean})$$

and the 95% confidence interval (95% CI) procedure were executed to determine significant and nonsignificant differences among treatment means (control would be considered a treatment) chlorophyll content and fresh weight response variables. Letters were assigned to treatment means to report significant and nonsignificant differences among means (means followed by the same letter are not significantly different according to 95% confidence level procedure). Excel was used to store raw data and RStudio was used to perform statistical analyses.

RESULTS

Plant growth and appearance

Overall, the plants that received GRO - the control treatment, outperformed the plants that received frass and vermicompost leachate as the treatment in terms of weight and chlorophyll levels.

The harvested samples were plotted on jitter plots as data points to visualize the distribution of plant weight (g) and chlorophyll levels (µg /cm^2) (see Figure 3). Control group had the highest average plant weight and chlorophyll levels, followed by FE where it experienced considerable stunted growth and considerable loss of chlorophyll levels , then lastly VL, with considerable stunted growth and more loss of chlorophyll levels than FE. See Figure 8 for a visual reference.

Fresh weight

The difference in plant harvest fresh weight (g) for all three treatment groups was significantly different from each other, though the difference between vermicompost and frass was minimal (Table 4). The control (mean = 6.39 g, lower CI = 5.68, upper CI = 7.09) had the highest mean fresh harvest weight, surpassing vermicompost (mean = 0.34 g, lower CI = 0.28, upper CI = 0.40), which surpassed frass (mean = 0.12 g, lower CI = 0.09, upper CI = 0.14) (see Table 7).

Figure 4 contains all collected data points for fresh weight that further illustrates the results. The treatment type is on the x-axis and the fresh weight in grams is on the y-axis. On the Scatterplot, the average weight for each treatment is indicated by the solid blue datapoint, which demonstrates the dramatic difference between the average weight of the control group and the two treatment groups.

Chlorophyll levels

The difference in chlorophyll concentration (μg/cm^2) for all three treatment groups was significantly different from each other (Table 3). The control (mean = 56.44 μg/cm^2, lower CI = 53.71, upper CI = 59.18) had the highest mean, surpassing frass (mean = 18.92 μg/cm^2, lower CI = 17.50, upper CI = 20.34), which surpassed vermicompost (mean = 5.64 μg/cm^2, lower CI = 5.26, upper CI = 6.03) (see Table 6). Figure 5 contains all collected data points for recorded chlorophyll level, separated by treatment type. The treatment type is on the x-axis and the chlorophyll level is on the y-axis. On the Scatterplot, each treatment group has a distinct range of chlorophyll levels with some overlap. The control had the highest level of chlorophyll, followed by FE, and then VL, all with normal data point distribution. The average chlorophyll level of each group is indicated by the solid red datapoint, which shows a difference in chlorophyll levels amongst all three experimental groups.

DISCUSSION

The objective of this research project was to perform a rudimentary examination of frass and vermicompost as potential alternatives to synthetic fertilizer in hydroponics for food production. The results from this experiment did not support FE and VL as standalone fertilizers for food production in an NFT setting. However, frass and vermicompost may still have the potential to boost plant growth (Beesigamukama et al., 2020) and plant immunity (Barragán-Fonseca et al., 2022) as fertilizer supplements instead of fertilizer.

Frass and Vermicompost Leachate as a Potential Hydroponic Fertilizer Supplements

Agricultural workers have long relied on synthetic fertilizers for a myriad of reasons including low cost (Wang et al., 2018) and its effectiveness in plant growth (Khaliq et al., 2006). However, environmental concerns arise with the production and use of synthetic fertilizer (Kanter, 2018; Sun et al., 2018), and the research on organic liquid fertilizer for hydroponics is increasing (Bergstrand, 2022; Xie et al., 2022; Bamdad et al., 2022; Phibunwatthanawong and Riddech, 2019). FE and VL as complete hydroponic fertilizer treatment failed reach similar levels of growth compared to synthetic fertilizer based on the chlorophyll levels and the weight of the *B. rapa* ssp. *chinensis* (Figures 6, 7, and 8). In this experiment, mealworm frass displayed statistically higher results in terms of chlorophyll content than vermicompost leachate, but both were significantly lower than the control. Vermicompost leachate failed to enter the acceptable EC range for optimum *B. rapa* ssp. *chinensis* growth. The optimum EC was between 1.5-2.0, and the optimum pH range was 7.0 (Singh and Dunn, 2016). The highest EC value recorded for VL was

1.44, which is 0.06 units short of the minimum acceptable value at 1.5, and the pH was on average 7.22. For comparison, the highest EC value recorded for FE was 2.22 and 2.65 for GRO; average pH for FE and GRO respectively are 8.13 and 6.90 (see table 8 for more details).

This experiment leaves room for research projects such as comparing the effectiveness of frass from other insects (e.g., black soldier fly larvae) as a novel source of hydroponic fertilizer. A great interest would be to use different frass types produced by different insects that are used for livestock animal feed, such as poultry mentioned earlier, in agriculture.

Research on repurposing insect frass ensures an increase in agricultural sustainability and can even potentially improve crop yield (Beesigamukama et al., 2020) and plant immunity (Barragán-Fonseca et al., 2022). Additionally, future studies can aim to study insect frass and vermicompost as a hydroponic nutrient supplement as opposed to a complete replacement of hydroponic fertilizer. In fact, a hydroponic study on tomato growth using vermicompost tea by Arancon et al. (2019) concluded that vermicompost's nutritional composition is not enough to cause substantial increases in yield by itself. However, trace amounts of plant growth hormones and humic acids in the vermicompost paired with synthetic fertilizers can lead to an increase in yields. The amount of synthetic mineral nutrients needed can be reduced if amended with low concentrations of vermicompost, which leads to significant reduction in production cost and an increase in agricultural sustainability (Arancon et al., 2019).

Pitfalls and Possible Improvements

There were limitations in this research project that could have influenced the results for this experiment. The lack of NFT system replication, potential concerns about the greenhouse environment such as high heat and exposure to pests, amongst other issues will be addressed in the following sections, along with methods that can improve or mitigate these issues in future experiments.

Replication and Pseudoreplication

This experiment attempted to eradicate any obvious biases in the experimental design. However, there are only three NFT systems that can be run simultaneously at any time because there are only three NFT frames that can support each 7-trough growing channel system. There was also a lack of adequate space that could ideally support multiple systems which would allow for replications necessary for this project. Combining the aforementioned concerns with the varying indoor temperatures (see section below), blocking the experiment by time would not be feasible. If the experiments were to be repeated consecutively with each run counted as replicate, they should not be grouped as the same data pool to avoid pseudoreplication concerns (Hurlbert, 1984). To mitigate the issue of pseudoreplication if this experiment were to be recreated, one must have adequate spacing, and all materials needed that can support all systems and their respective replicates.

The Greenhouse

Theoretically, a climate-controlled greenhouse should provide all the necessary protection against elements and confounding factors that can impact plant production. In the case of this experiment, however, the structure of the greenhouse was compromised and remained so throughout the duration of the experiment. This caused the greenhouse to have multiple entryways for pests such as whiteflies, mice, rats, snakes, ants, and cabbage loopers to have access to the *B. rapa* ssp. *chinensis*. The pests did not cause any visible damages to the crops, but the potential negative impacts of their presence in the greenhouse should not be dismissed. Additionally, the greenhouse did not provide complete protection against high temperatures from heat waves that are common during the late summer to early fall period in Southern California. Despite the presence of cooling cell walls, the highest recorded ambient temperature indoors reached 93 degrees Fahrenheit in the mornings. The heat impacted *B. rapa* ssp. *chinensis* germination, even with its heat tolerant trait. The number of seedlings that germinated was enough for an experimental run (N= 126 plants per treatment), however, the negative impacts the high temperature had on the germinated seedlings should be considered when viewing the experimental results.

Water

The baseline water pH obtained from the chemical analysis report from Denele Analytical, Inc. (Turlock, CA) was noticeably different than when the water was tested on-site with our pH and EC probe. According to annual water quality reports from the 2022 Water Quality Report ("2022 Consumer Confidence Report", 2022), Pomona ground water was sampled from January 2021 - December 2021. The average pH for Pomona groundwater was reported to be 7.43, with the minimum to maximum range varying from 6.69 to 8.09. Denele's pH reading was close to the maximum range of groundwater in Pomona. The chemical analysis report from Denele Analytical indicated that the pH of the greenhouse tap water was at 8.1; The pH of the water based on the

HANNA pH and EC probe was hovering between 7.2-7.4. The difference in pH could potentially affect plant growth (Islam et al., 2014; Neina, 2019), which may affect the growth of *B. rapa* ssp. *chinensis* in this experiment and subsequently the results.

FE and VL formulation

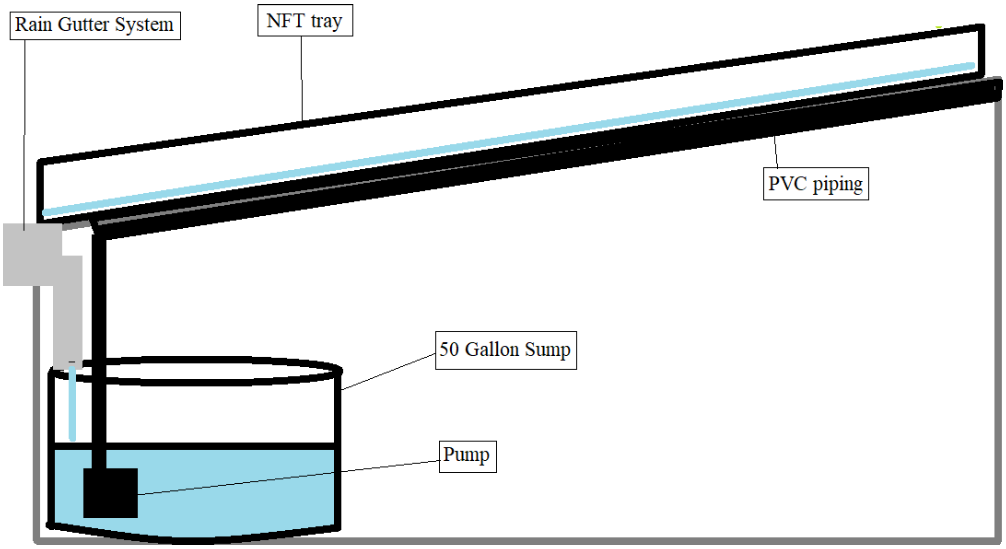
There might have been discrepancies between FE and VL solutions when the solutions were made, as the experiment called for all treatments to be identical to each other in terms of EC, and pH (see Table 8). Producing the nutrient solution involved measuring the EC and pH of all treatment solutions after they have been made. However, the EC and pH did not remain consistent throughout the duration of the experiment (see Table 8). As stated in the Methods section, VL failed to reach the minimum viable EC for proper *B. rapa* ssp. *Chinensis* growth at the beginning and remained so throughout the entire duration of the experiment. FE reached a pH of 7 right at the beginning of producing the treatment. However, the pH quickly rose to an average of 8.13, with the highest value being 8.32. This upswing in pH is likely what caused stunted plant growth (Islam et al., 2014; Neina, 2019) despite meeting the EC requirements for optimum bok choy growth.

CONCLUSION

The effectiveness of frass and vermicompost as sources of plant-ready nutrients in bok choy production and its potential to replace commercial synthetic hydroponics nutrient solutions was evaluated. To determine this potential, an NFT experiment comparing three hydroponic nutrient solution treatments was conducted with one replication for each of the following treatments: frass leachate (FE), vermicompost leachate (VL), and commercial synthetic nutrient solution (GRO). The fresh weights and chlorophyll levels of the *B. rapa* ssp. *Chinensis* were recorded and compared between treatments. The weight of the harvested plants was statistically different from one another between the treatments, with VL resulting in a higher weight than FE. However, both VL and FE weighed statistically less than the control bok choy fresh weight. Interestingly, chlorophyll had differences amongst all three treatments that were significant, and the plants that had the most chlorophyll to least are the control, FE, then lastly, VL. This project aimed to diversify the sources of nutrient solutions in hydroponic systems by introducing mealworm frass and vermicompost leachate, which are cheap and readily available resources, as novel sources of hydroponic fertilizer. The results of this research project reached the conclusion that vermicompost leachate and mealworm frass leachate are unable to serve as a complete replacement for synthetic hydroponic fertilizer. This project nonetheless promotes the use of waste by-products in the insect rearing industry to create an environmentally friendly source of plant-ready nutrients that can be used in hydroponic food production.

FIGURES

Figure 1: Nutrient film technique system

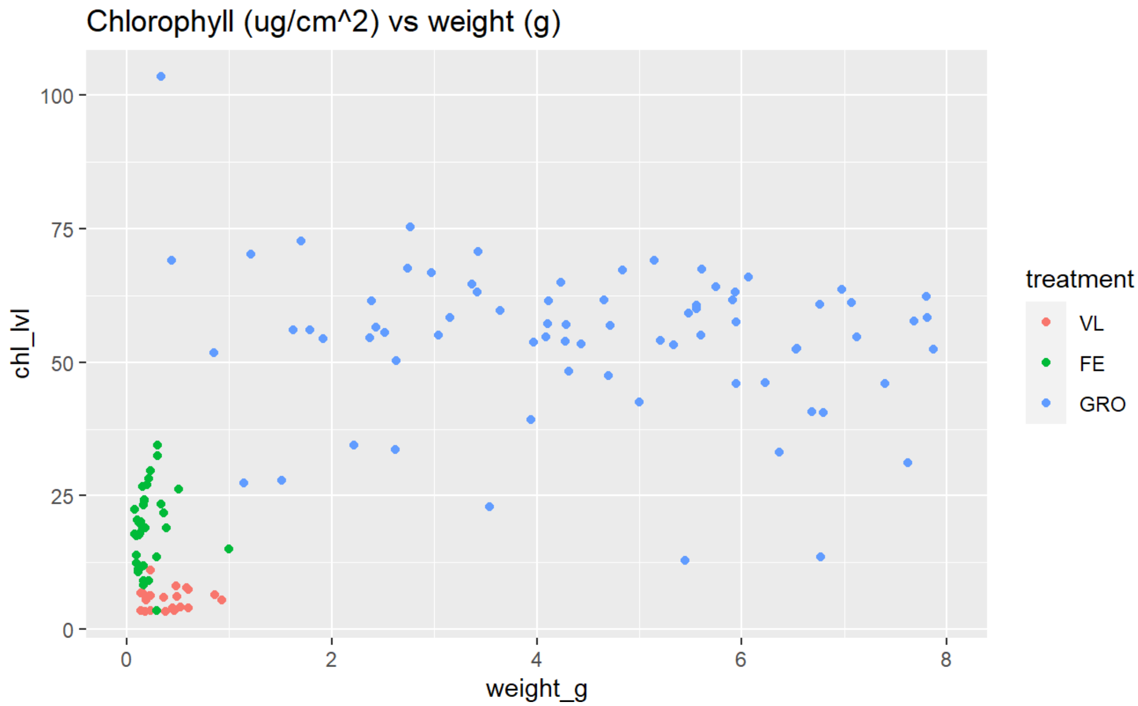


Note: Side-view a nutrient film technique (NFT) system.

Figure 2: Photo of the nutrient film technique system

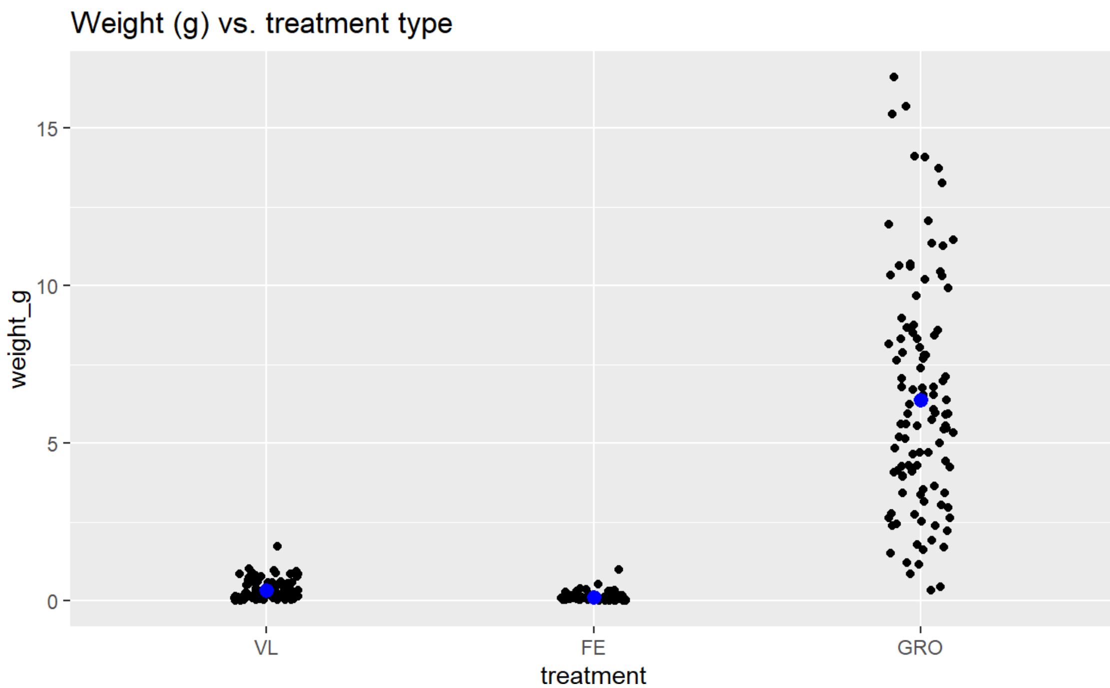
Note: Photo of the nutrient film technique system with Grow More (GRO) treatment in the Cal Poly Pomona research greenhouse.

Figure 3: Scatterplot of all data points



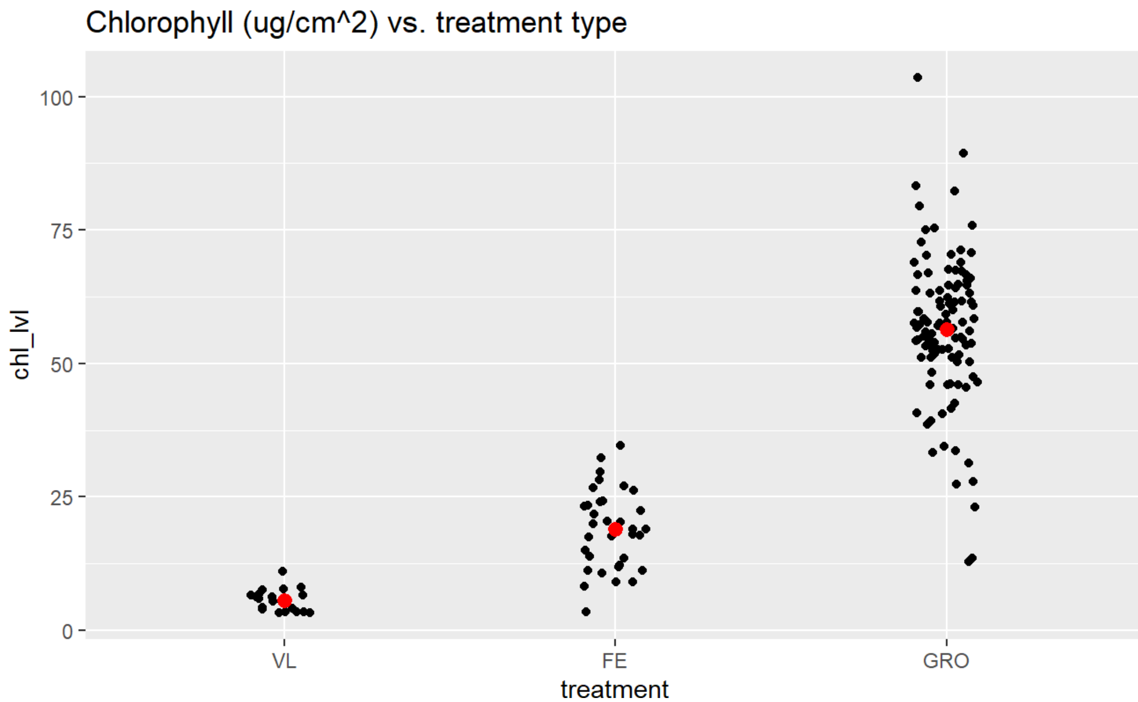
Note: Scatterplot of chlorophyll level (µg/cm^2) vs weight (g), treatment differentiated by color, produced with RStudio. The blue datapoints represent the control treatment – Grow More (GRO), green represents the frass treatment (FE), and red represents the vermicompost leachate treatment (VL).

Figure 4: Scatterplot of fresh weight



Note: Scatterplot of weight (g) vs. treatment type, blue dot as the mean weight in grams, produced with RStudio. The treatment groups from left to right are the following: VL for vermicompost leachate, FE for frass leachate, and GRO for the control – Grow More.

Figure 5: Scatterplot of chlorophyll level



Note: Scatterplot of chlorophyll level (µg /cm^2) vs. treatment type, red dot as the mean, produced with RStudio. The treatment groups from left to right are the following: VL for vermicompost leachate, FE for frass leachate, and GRO for the control – Grow More.

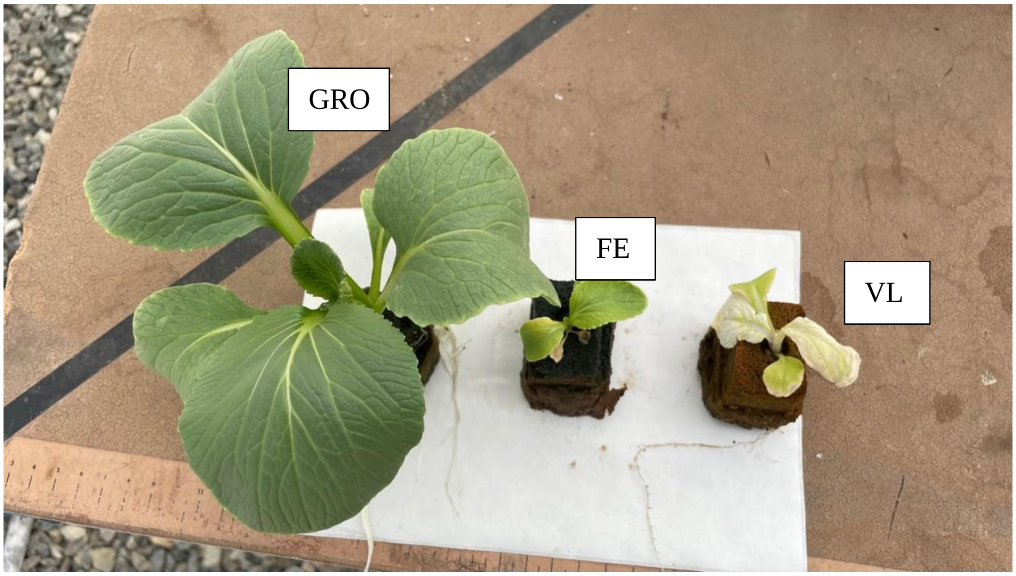
Figure 6: Photo of plants with FE

Note: Overhead view of the harvested plants that received frass leachate (FE) as treatment. The color is a light green color with many experiencing stunted growth.

Figure 7: Photo of plants with VL

Note: Overhead view of the harvested plants that received vermicompost leachate (VL) as treatment. The color is a pale green to pale yellow color with many experiencing slight stunted growth.

Figure 8: Side by side comparison of all treatments



Note: Overhead view of harvested bok choy plants side-by-side from left to right: Grow More (GRO), frass leachate (FE), and vermicompost leachate (VL).

TABLES

Table 1. Knop's standard nutrient solution.

Compound	g/1
KNO_3	0.2
$Ca(NO_3)_2$	0.8
KH_2PO_4	0.2
$MgSO_4 \bullet 7H_2O$	0.2
$FePO_4$	0.1

Note: Knop's standard nutrient solution with the compound names on the left column (Jones, 1982).

Table 2. Example of a modified Hoagland's nutrient solution

Compound	Concentration
$Ca(NO_3)_2.4H_2O$	236.1 g/l
KNO_3	101.1 g/l
KH_2PO_4	136.1 g/l
$MgSO_4.7H_2O$	246.5 g/l
Trace elements (make up to 1 l)	
H_3BO_3	2.8 g
$MnCl_2.4H_2O$	1.8 g
$CuSO_4.5H_2O$	0.1 g
$ZnSO_4.7H_2O$	0.2 g
$NaMoO_4$	0.025 g
FeEDTA (200 ml)	
EDTA	2.08 g
$FeSO_4$	1.56 g
KOH	11.22 g

Note: Example of a modified Hoagland's nutrient solution for modern hydroponic use (Malhotra et al., 2014).

Table 3. Baseline Greenhouse tap water test results table

Water Test					
Analyte	Result	Units	meq/L	lbs/ac in.	lbs/ac ft.
pH	8.1	Units			
Electrical Conductivity (EC)	0.81	mmhos/cm			
Soluble Salts	5.18	mg/L			
Nitrate Nitrogen	4.7	ppm		1.06	12.7
Nitrate	20.8	ppm		4.69	56.2
Bicarbonate	21.4	ppm	3.5	48	576
Calcium	7.77	ppm	3.89	17.5	210
Magnesium	21.3	ppm	1.76	4.8	57.6
Sodium	55.6	ppm	2.46	12.85	150
Potassium	7.37	ppm	0.189	1.66	19.9
Boron	173	ppm		0.387	4.65
Chloride	65.8	ppm	1.85	14.8	178
Sulfate	136	ppm		30.5	366
Sodium Absorption Ratio	1.44				

Note: The chemical analysis of the Greenhouse tap water before any alterations or additions of the hydroponic nutrient solutions. Results were obtained on July 19th, 2022 (Denele Analytical, Inc., Turlock, CA, USA).

Table 4. Baseline vermicompost test results table

Vermicompost Complete Manure Test

Analyte	Method Ref.	Result	Units	Lbs/Ton as Rec'd
Moisture (H2O)	SM2540B	59.7	%	N/A
pH (pH)	SM4500-H+ B-2000	6.1	Units	N/A
Electrical Conductivity (EC)	SM 2510 B-1997	18.4	mmhos/cm	N/A
Boron (B)	EPA 200.7	37.6	mg/L	0.03
Zinc (Zn)	EPA 200.7	179	mg/L	0.144
Iron (Fe)	EPA 200.7	4820	mg/L	3.89
Manganese (Mn)	EPA 200.7	192	mg/L	0.155
Copper (Cu)	EPA 200.7	90.9	mg/L	0.073
Organic Matter (OM)	S - 9.20	76.6	%	617
Potassium (K)	EPA 200.7	0.905	%	7.29
Total Phosphorus (TP)	SM 4500P E	0.433	%	3.49
Sodium (Na)	EPA 200.7	0.15	%	1.21
Calcium (Ca)	EPA 200.7	1.62	%	13.1
Magnesium (Mg)	EPA 200.7	0.62	%	5
Sulfur (S)	Combustion	0.56	%	4.51
Total Nitrogen (TN)	Combustion	3.51	%	28.3
Soluble Salts (SALT-SOL)	Calculation	11800	mg/L	9.49
C:N Ratio (C:N)	Calculation	12:01	N/A	N/A

Note: The manure test of the vermicompost before any alterations or additions of water to produce the hydroponic nutrient solutions. Results were obtained on July 19th, 2022 (Denele Analytical, Inc., Turlock, CA, USA).

Table 5. Baseline frass test results table

Frass Complete Manure Test

Analyte	Method Ref.	Result	Units	Lbs/Ton as Rec'd
Moisture (H2O)	SM2540B	12.1	%	N/A
pH (pH)	SM4500-H+ B-2000	5.7	Units	N/A
Electrical Conductivity (EC)	SM 2510 B-1997	7.51	mmhos/cm	N/A
Boron (B)	EPA 200.7	14.8	mg/L	0.026
Zinc (Zn)	EPA 200.7	116	mg/L	0.204
Iron (Fe)	EPA 200.7	375	mg/L	0.659
Manganese (Mn)	EPA 200.7	186	mg/L	0.327
Copper (Cu)	EPA 200.7	18.7	mg/L	0.033
Organic Matter (OM)	S - 9.20	93.2	%	1640
Potassium (K)	EPA 200.7	1.64	%	28.8
Total Phosphorus (TP)	SM 4500P E	1.31	%	23
Sodium (Na)	EPA 200.7	0.024	%	0.422
Calcium (Ca)	EPA 200.7	0.203	%	3.57
Magnesium (Mg)	EPA 200.7	0.651	%	11.4
Sulfur (S)	Combustion	0.24	%	4.22
Total Nitrogen (TN)	Combustion	3.03	%	23.3
Soluble Salts (SALT-SOL)	Calculation	4810	mg/L	8.45
C:N Ratio (C:N)	Calculation	18.1	N/A	N/A

Note: The manure test of the vermicompost before any alterations or additions of water to produce the hydroponic nutrient solutions. Results were obtained on July 19th, 2022 (Denele Analytical, Inc., Turlock, CA, USA).

Table 6. 95% CI summary of chlorophyll levels of *B. rapa* ssp. *Chinensis*

The effect of VL, FE, and GRO on chlorophyll levels (μg/cm^2)			
Treatment	**Mean**	**Lower CI**	**Upper CI**
VL	5.65 c^z	5.26	6.03
FE	18.92 b	17.50	20.34
GRO	56.44 a	53.71	59.18

Note: Chlorophyll level (μg/cm^2) of *B. rapa* ssp. *Chinensis* treated with vermicompost leachate (VL), frass leachate (FE), and Grow More (GRO). The control (mean = 56.44 μg/cm^2, lower CI = 53.71, upper CI = 59.18) had the highest mean, surpassing frass (mean = 18.92 μg/cm^2, lower CI = 17.50, upper CI = 20.34), which surpassed vermicompost (mean = 5.64 μg/cm^2, lower CI = 5.26, upper CI = 6.03).

^{z}Means followed by the same letter are not significantly different according to 95% confidence level procedure.

Table 7. 95% CI summary of *B. rapa* ssp. *Chinensis* fresh weight (g)

The Effect of VL, FE, and GRO on weight (g)			
Treatment	**Mean**	**Lower CI**	**Upper CI**
VL	0.34 b[y]	0.28	0.40
FE	0.12 c	0.09	0.14
GRO	6.39 a	5.68	7.09

Note: This is a 95% CI table summarizing the fresh weight (g) of *B. rapa* ssp. *Chinensis.* The treatment groups from top to bottom are the following: VL for vermicompost leachate, FE for frass leachate, and GRO for the control – Grow More. The control (mean = 6.39 g, lower CI = 5.68, upper CI = 7.09) had the highest mean fresh harvest weight, surpassing vermicompost (mean = 0.34 g, lower CI = 0.28, upper CI = 0.40), which surpassed frass (mean = 0.12 g, lower CI = 0.09, upper CI = 0.14).

[y]Means followed by the same letter are not significantly different according to 95% confidence level procedure.

Table 8. EC and pH of the nutrient solutions.

Treatment	FE		VL		GRO	
	EC	**pH**	**EC**	**pH**	**EC**	**pH**
Min	1.97	7.94	1.23	6.97	1.86	6.76
Max	2.22	8.32	1.44	7.47	2.65	7.03
Average	2.10	8.13	1.34	7.22	2.26	6.90

Note: Electroconductivity (EC) and pH range for the treatment nutrient solutions – frass leachate (FE), vermicompost leachate (VL), and lastly Grow More (GRO), the control.

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