OPTIMIZING CONTROLLED-RELEASE FERTILIZER FOR LETTUCE AND MIZUNA GROWN ON THE INTERNATIONAL SPACE STATION

by

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I dedicate my work to the future generations that will make Mars green

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ABSTRACT

Astronaut diets on the International Space Station (ISS) depend on resupplied packaged food. However, missions to Mars of 3-5 years will not accommodate re-supply. In addition, many human macro and micronutrients degrade during long-term storage. Thus, growing nutritional plants aboard ISS is essential for providing astronauts with fresh, healthy produce. NASA is usingan experimental vegetable- production unit called VEGGIE to grow fresh salad crops aboard ISS to provide astronauts with healthy diets. VEGGIE is a small plant-growth chamber designed as a garden for astronauts that is low in mass and has a low power requirement. Veggie is equipped with light-emitting diodes (LEDs) but is exposed to the ISS cabin environment. Plants are grown with roots in a baked-ceramic substrate (arcillite) incorporating controlled-release fertilizer (Nutricote) and wicks delivering water by capillary action from a reservoir.

The fertilizerprills release nutrients into arcillite slowly over time. Different controlled-release types have the same amount of fertilizer but release it over different time periods. The Purdue Mitchell lab in collaboration with NASA is testing growth of salad crops within VEGGIE analogs under ISS-like environments in a growth chamber. Specifically, we are evaluating effects of different controlled-release fertilizer treatments as well as different substrate particle sizeson "cut-and-come-again" harvest scenarios, comparing productivity and quality of Lettuce as well as anAsian salad crop called Mizuna.

ISS environments being mimicked include temperature: 24/21°C D/N, CO_2: 2800 PPM D/N, RH: 45-50% D/N, and photoperiod: 16hours.Arcillitemedium contained one oftwo different fertilizer mixes: 7.5g18-6-8 T 70 + 7.5g 18-6-8 T100, or 7.5g18-6-8 T70 +7.5g 18-6-8 T180fertilizer/liter medium. LED Light treatment provides atotal PPFDof 330µmol $m^{-2}s^{-1}$ PAR; with 270µmol $m^{-2}s^{-1}$Red(R), 30µmol $m^{-2}s^{-1}$Blue (B), and 30µmol $m^{-2}s^{-1}$Green (G). Plants are grown under those conditions for 8 weeks, and harvested three times at 28, 42, and 56 days from planting. At each harvest, yield parameters as well as tissue mineral content have been measured for optimum fertilizer treatment selection.

Lettuce and Mizuna plants grown in a mix of 100% fine substrate particles (Profile) and fertilizer treatment of 50% T100:50%T70 had the higher yield as well as nitrogen contentcompared to those grown in 50%T180:50%T70. Growing mizuna plants in 100% profile resulted in higher shoot fresh weight; although no significant differences occurred for shoot dry weight. In addition, there was no significant interaction between substrate and fertilizer, which is reported by other research as one of the advantages of using controlled-release fertilizer

CHAPTER 1. LITERATURE REVIEW

The increasing demand for growing healthy, nutritious produce and advancements in controlled-environment technology have promoted the idea of growing fresh produce for the crew aboard the international space station (ISS). Plants are the major nutrient resource for human kind, and the only organisms that could form a bioregenerative support system with humans. During short-term missions, produce is delivered to the astronauts via re-supply trips. However, in long-term missions beyond low earth orbit, this will not be accessible, and growing plants on board will be necessary. In addition, many macronutrients and micronutrients degrade with increasing storage time (Zwart et al., 2009). Hence, establishing a protocol for on-board vegetable production ensures food security for astronauts, diverse crew diet, and provides proper nutrients to influence their performance and enhance their morale (Massa et al., 2019). Numerous questions should be answered before establishing a working protocol for growing plants in space. These questions include, but are not limited to, choosing candidate plants, optimizing growing conditions, using appropriate technology, reducing expected cost, and finally overcoming unfavorable conditions such as super-elevated CO_2 and radiation.

1.1 Establishing bioregenerative life-support systems

The idea of establishing a bioregenerative life-support system between humans and plants started before the idea of space agriculture. Plant photosynthesis generates carbohydrates (CH_2O) and oxygen (O_2) while using carbon dioxide from human respiration (Galston, 1992; Ferl et al., 2002). In addition, water provided to plants could be condensed after plant transpiration as clean water that could be recycled (Wolverton et al., 1983). Algae, specifically *Chlorella pyrenoidosa*was, was the preferred organism for bioregenerative studies in the1950s and 60s (Sorokin and Myers, 1953; Krauss, 1962; Eley and Myers, 1964; Miller and Ward, 1966). Algae were a good candidate for O_2 production and CO_2 removal studies (Gouleke and Oswald, 1964; Miller and Ward, 1966; Taub, 1973). However, with the rise of interest in long-term space missions there also were growing interests in palatable foods (Krauss, 1962; Karel et al., 1985; Nakhost et al., 1987). Several groups started bioregenerative life-support systems all around the world: Russia (Gitelson et al., 1976; Salisbury et al., 1997), Canada (Grodzinski, 1992; Stasiak et al., 1998), Europe (Skoog, 1987;

Gerbaud et al., 1988; Daunicht and Brinkjans, 1992) and Japan (Nitta and Yamashita, 1985; Oguchi et al., 1987). In early 1980s The National Aeronautics and Space Administration (NASA) relaunched bioregenerative research via the controlled ecological life support systems (CELSS) Program (Moore et al., 1982). CELSS focused mainly on nutrition, food and waste processing, food production, closed system ecology, and system engineering (Mason and Carden, 1982), crop selection, harvest index, and nutritional needs (Hoff et al., 1982; Tibbitts and Alford, 1982). Findings from CELSS in different universities were tested in the Biomass Production Chamber (BPC) at NASA's Kennedy Space Center (KSC) from the 1988 to1990. However, yields from BPC were lower compared to open chambers (Wheeler et al., 1996). Hereafter, the NASA bioregenerative life-support program became known as the Advanced Life Support Program (ALSP). In 1996, Edeen et al., showed that 11 m^2 of wheat grown at high temperature and high light could provide sufficient oxygen for one human.

1.2 Veggie plant-growth system

Veggie is a small plant-production system on ISS that was first introduced in 2014 by Orbital Technologies Corp. (ORBITEC, Madison, WI), and tested at NASA's Kennedy Space Center in Florida (Morrow et al., 2005; Morrow and Remiker, 2009). Dimensions of Veggie`s base plate are29.2 cm wide by 36.8 cm deep, and maximum available shoot length does not exceed 47 cm (Massa et al., 2017). Veggie is fully lighted by Light-emitting diodes (LEDs): red (630 nm), blue (455 nm) and green (530 nm) either manually or automatically (Massa et al., 2016). Seeds are planted in six plant pillows containing calcined clay and controlled-release fertilizers, on a 2L root matreservoir designed for passive diffusion of water into the pillows. Veggie was designed to consider low power, low mass, and low crew time to operate it (Fig. 1) (Massa et al., 2017).

1.3 Selection of plant species for space agriculture

Determining the best plants to grow in space started during a symposium in1958 at Wright Patterson Air Force Base. In that symposium, the first list of potential candidate crops for space missions was developed. These cultivars share similar standards such as compact size, high productivity, tolerance to osmotic pressure, and response to low light intensity. This list contained Lettuce, Chinese cabbage, cabbage, Cauliflower, kale, turnip, Swiss chard, endive, dandelion,

radish, New Zealand spinach, tampala, and sweet potato (Boeing Comp., 1962; Gouleke and Oswald, 1964). These crops differ in their nutritional profile, horticultural characteristics, and palatability. In addition, they should be tested for different parameters essential for space agriculture such as ease of cultivation, growth-system requirements, stress tolerance, nutritional value, reliability, speed of germination, rapid growth, and low microbial level (Anderson et al., 2017). Plants react differently to the space environment. Testing the effects of ISS temperature, humidity and elevated level of CO_2 on eight leafy-green crops in controlled growth chambers revealed different effects on different cultivars (Massa et al., 2015). These eight cultivars were mizuna, Chinese cabbage cv. Tokyo Bekana, Swiss chard cv. Rhubarb, Bull's Blood beet, green leaf lettuce cv. Waldmann's Dark Green, red romaine lettuce cv. Outredgeous, Spinach cv. Tyee, and spinach cv. Flamingo. Characteristics tested included levels of anthocyanins, antioxidant (ORAC-fluorescein) capacity, lutein, zeaxanthin, Vitamin K, growth rate, yield, and mineral content. Sensory evaluation showed that Chinese cabbage, lettuce, Swiss chard and mizuna are appropriate candidates for pick-and-eat scenarios on ISS (Massa et al., 2015).

1.4 Optimizing nutrient content of space-grown plants

Fruit and vegetables are vital nutrient sources. Prepackaged food does not contain the amazing complex of vitamins, bioavailable phytonutrients, and minerals included in plant tissues. The interaction between these mixtures and human body provide combined effect that cannot be supplemented by any form of isolated supplement (Liu, 2003).

1.4.1 Effect of long-term storage on nutrient content of stored produce

Dietary supplements increase the risk of toxicity and show limited effects improving vitamin and mineral concentrations in human tissue and blood serum (Liu, 2003). Non-significant differences existed in macronutrient and micronutrient contents between both ground and low-earth orbit packaged food after 880 days of storage. However, long-term storage (> 880 days storage) led to significant decreases in macronutrients and micronutrients compared to controls (Zwart et al., 2009). Oxidization of sulfur in plants could lead to amino acid oxidization, which leads to imbalanced acid-base content in human blood (Giovanelli, 1987; Lane et al., 2013).

1.4.2 Effect of space flight on nutritional status of astronauts

During both short and long-duration space flights, undesirable changes occurred in overall nutritional status of the astronaut bodies (Smith et al., 2005). These changes are possibly related to the imbalance between energy expenditure and intake. Energy expenditure was unaffected, but energy intake showed a 30-40% decrease below normal levels (Smith et al., 2001; 1999; Stein et al., 1999; Lane et al., 1997). Several critical changes occurred on astronauts bodies during long-term space travels (128–195 d) such as oxidative damage, bone loss, and compromised vitamin D (Smith et al., 2005). Providing fresh produce with optimum nutrient content to the astronauts likely would help reduce or prevent these problems.

Bone resorption or bone break down by osteoclasts is common in astronauts during space flights (Teitelbaum, 2000). Bone resorption could lead to 250mg/day loss of bone calcium (Smith et al., 1999). Ionized calcium decreased (P=0.06) in astronaut bodies after long-term space flight (Smith et al., 2005). Possibly, the absence of resistance to muscle movement in microgravity is the major reason for bone resorption (Nabavi et al., 2011). However, after the development of the Advanced Resistive Exercise Device (ARED) bone loss is not much of a problem these days (Loehr et al., 2011)Both magnesium and phosphorus level in astronaut's body tissues were impacted by space flight, but urine analysis showed non-effect on magnesium level (Smith, et al. 2005; 2015). Phosphorus could form phytic acid in plant tissue and lead to calcium phytate, which causes calcium loss in human bodies (Nielsen, 1996). However, there is not enough evidence for negative impact of enhanced phosphorus dietary on the human body. Long-term space flight and exposure to elevated radiation levels impose iron storage in astronauts' tissues, which increases bone loss and oxidative stress (Zwart et al., 2013). Iron-enhanced food raised both iron accumulation and oxidative stress in human bodies (Pouraram et al., 2012). Potassium is often limited in different food types available for astronauts (Lane and Shoeller, 2000), dietary sodium intake (from pre-packaged foods) for astronauts is 3000 mg/day, so sodium content of produce must be monitored to avoid exacerbation of body stress (Lane et al., 2013). Plants produce carotenoids such as lutein and zeaxanthin, which play a role in photo protection against light. These molecules are used in human eyes to protect photoreceptors from radiation damage (Demmig and Adams, 2013). Yet, optimizing nutrient content of fresh produce grown on-board could help avoid several problems related to the effect of the space environment on astronaut's bodies.

1.5 Optimizing growing conditions for space-grown plants

1.5.1 Light conditions

Light-emitting diodes (LEDs) are the most effective lightning technology for crop growth on long-duration spaceflight missions for several reasons: First, LEDs have extended lifetimes compared to traditional lighting sources such as high-intensity discharge (HID) lamps (Bourget, 2008). Second, LEDs permit investigation of optimal spectral ratios to improve plant growth and development (Nelson and Bugbee, 2014; 2015). Third, LEDs do not induce heat stress with close-canopy lightning, which leads to: (1) lower power consumption with the same level of photon flux density (PPFD); (2) growing crops in small spaces on spacecraft (Poulet et al., 2014).

Manipulating spectral ratios influenced growth and development of several plant species. For instance, 24% green florescent light led to a significant increase of fresh weight, dry weight, and leaf area of lettuce compared to 24% red and blue (Kim et al., 2004). Red light accompanied with 10% blue light promoted dry weight of lettuce, spinach, and radish (Yorio et al., 2001). Blue light promoted antioxidant content of leafy greens, whereas red light increased stem elongation (Li and Kubota, 2009). Moreover, manipulating various spectral ratios led to significant effects on several physiological characteristics. Spectral ratios influenced various rates of intercellular CO_2 concentration (Ci), stomatal conductance (gs), and overall photosynthetic rate (Kim et al., 2005). Spectral ratio also influenced the production of carotenoids, including lutein, a compound that plays a role in human eye protection against high radiation (Demmig-Adams and Adams, 2013). As well these ratios affected epoxidation and de-epoxidation of xanthophylls (Ruban et al., 1994). Changing spectral ratios resulted in different ratios of stem elongation, phytochemical concentration and stem elongation in lettuce (Mitchell, 2015; Li and Kubota, 2009; Kim et al., 2004; 2005).

1.5.2 Effects of elevated CO_2concentrations

Plants grow normally at ambient carbon dioxide (CO_2) concentrations of 300-400$\mu molmol^{-1}$. However, in spacecraft or the space station, CO_2 exists at super-elevated concentrations.

Plant's ability to acclimate to high-elevated CO_2 is a determining factor in crop selection for growth in space. Different plant species react differently to elevated levels of CO_2. Even though elevated CO_2 concentrations may cause a temporary increase in photosynthetic activity, overtime

it typically reduces photosynthetic activities (Bugbee et al., 1994; van Berkal, 1984; Bowes, 1991). Higher rates of photosynthetic activities lead to higher accumulation of carbohydrates in photosynthesizing leaves (Stitt, 1991). Excessive carbohydrate accumulation affects *rbcS* genes, which code for ribulose bisphosphate carboxylase oxygenase (RuBisCO), the CO_2 carboxylation enzyme (Sage et al., 1989; Van Oosten and Besford, 1994).

Elevated CO_2 may lead to stomata aperture reduction and decrease in transpiration, which consequently will decrease water uptake and nutrient movement into the plant. Lack of the nutrient acquisition from the root zone leads to reduced plant aptitude to acclimate to elevated CO_2 (McDonald et al., 2002). In elevated CO_2 conditions, leaf chlorotic spots could be a response to starch accumulation and mineral deficiencies (Ehret and Joliffe, 1985 Tripp et al., 1991; van Berkal, 1984). Under high levels of CO_2 leaf nitrogen content reduced in several plant species (Monje and Bugbee, 1998; Sage et al., 1989), and reduced K and Mn in tomatoes leaves during fruiting (Tripp et al., 1991). On the other hand, elevated CO_2 showed different effects on the yield of different crops. Tomatoes showed increased fruit development and reduced root growth in 1,000µmolmol^{-1} CO_2 (Tripp et al., 1991). Wheat exhibited reduced leaf and root development (Monje and Bugbee, 1998). Cucumber showed faster accumulation of biomass under elevated CO_2 conditions with no effect on total yield (Peet, 1986).

1.5.3 Selection of substrate

In general, plants need a water and nutrient-delivery system (NDS) to support their growth and development. Appropriate media for a space agriculture experiment should allow sufficient water-holding capacity, adequate nutrients, and should fulfill NASA-based standards of acceptable microbial content (Massa et al., 2013). Yet, in spacecraft, it also is essential to use NDS that fulfill operational and safety constraints (Stutte et al., 2011). Numerous approaches have been tested for producing plants in a reduced gravity environment, including porous membranes that use variable negative pressure or constant pressure (Wright et al., 1988; Koontz et al., 1990; Berkovich et al., 2002);Phenolic foam (Musgrave et al., 1997);Balkanine and Turface (Jones and Or 1999);Turface with porous tubes (Morrow et al., 1997); 1-2 mm Turface/Osmocote with porous tubes; solid substrate either manufactured or natural with capillary wicking from a reservoir (Kliss et al., 2000; Morrow et al., 2005; Morrow and Remiker, 2009); or sub-irrigation (Bingham et al., 1996; Goins

et al., 1997). Porous media were not successful, for reasons that are not yet clear, and it could involve an inappropriate supply of water and nutrients to plant roots in the microgravity environment (Jones and Or, 1998).

Even though recirculating hydroponic systems are valuable in ground studies (Wheeler et al., 1996). They are difficult to apply in spacecraft because of their large size (i.e., water volumes, pumps, tubes) (Wright et al., 1988; Peterson et al.,1991; Dreschel and Sager, 1989), and because the mass and volume of dilute nutrient solution required to get away from higher levels of containment (Stutte et al., 2011) would be prohibitively high.

Substrates were shown to attain similar moisture distribution to hydroponics (Monje et al., 2003).Solid substrate in the NDS is most preferable, especially for long-term space missions, as they can made mechanically reliable, simple, and allow sufficient water transfer to the root zone (Monje et al., 2003).Large-grain of substrate allows appropriate aeration but limits water uptake, and small-grain substrate allows appropriate water uptake but limits aeration (Casado, 2006). Grain size of substrate used for NDS in space studies ranged from 0.5 to 5 mm, and the most commonly used substrates are Arcillite with size 1–2 mm, and zeoponic with size 0.5–1 mm (Monje et al., 2003).

Arcillite less than 1-mm diameter is a suitable growth medium to use in space agriculture because of its inorganic nature (calcined clay), which does not promote microbial growth, and allows higher plant-growth rate compared to other media. In addition, it had uniform results when tested with three different lettuce cultivars. (Jones and Or 1999; Flemming et al., 2012).

1.5.4 Selection of fertilizer

Using slow-release fertilizer (SRF) and controlled-release fertilizer (CRF) improved nutrient concentrations in soils throughout the growing period (Handreck and Black, 2002). Polymer-coated fertilizers (PCF) are the most advanced controlled-release fertilizers and have been used widely with high-value field crops grown under high leaching conditions (Goertz, 1993). Yet, they were used with zero-leaching root zones of plants grown in space (Monje et al., 2003; Salisbury and Bugbee, 1985; Kochbaet al., 1990).

There are three basic mechanisms of release for PCF, osmotic pumping, convective release by coating disruption, and diffusion (Kaunisto et al., 2011; Shavivet al., 2003). Water-vapor pressure of the environment surrounding the PCF plays a major role in release rate as substrate with high water vapor pressure would lead to higher water uptake. Release pattern of certain fertilizer could be achieved by varying size of the small pellets known as prills (Kochba et al., 1994). In addition, various physical characteristics such as coating thickness and fertilizer granule radius vary within population of prills (Al-Zahrani, 1999; Du et al., 2004, 2008). Nevertheless, the majority of PCFs release most of their nutrient contents early, and the rate of release reduces over time, and among the primary macronutrients nitrogen (N) showed the highest release rate followed by magnesium (Mg) and finally phosphorus (P) (Broschat, 2005; Broschat and Moore, 2007; Du et al., 2006; Huett and Gogel, 2000). Manganese (Mn), molybdenum (Mo), and iron (Fe) showed less than 50% elease in some cases (Moore, 2007).The release period from PCF prills is divided into three different stages; first stage is lag phase where release rate is very slow; second stage is a linear phase where release rate increases; and last stage is decay phase where release rate declines (Shavivet al., 2003). Nutrient release rate from PCF is highly impacted by temperature. In contrast, nutrient release rate is unaffected by substrate type and texture, pH, and biological activity (Broschat, 2005; Kochba et al., 1990; Oertli and Lunt, 1962; Shibataetal., 1980).

Adams et al.(2013) studied the stability of release rate of three major PCFs: (1) Osmocote Plus (Grace Sierra Horticultural Products Co, Marysville, Ohio, USA), (2) Nutricote Total with Minor Nutrients (ChissoAsahi Fertilizer Company, LTD., Tokyo, Japan), and (3) Polyon Coated NPK Plus (Pursell Industries, Sylacauga, Alabama, USA). Adams et al. (2013) confirmed three main facts: First, nutrient release rates of all three PCFs were not impacted by water, moisture, and solid substrate status. Second, nutrient release of Nutricote (18-6-8) fertilizers (T100, T270 and T360) showed the highest delivery steady-state release rate at temperature of 20°C to 30°C. Third, variability of Osmocote was erratic (10%-40%) compared to Nutricote and Polyon at 10% and 5%, respectively.

1.6 Cut-and-come-again harvest method

It is very essential to develop harvest method to maximize productivity per volume on the spacecraft related to cultivar growing habit (Anderson et al., 2019). “Cut-and-come again” is a harvest method that allows multiple harvests with one time sowing and allows conservation of resources and speedy harvest. In addition, it saves astronauts time by expanding growth period using the same resources. Lettuce plants harvested by cut-and-come again method produced higher total yield compared to “one-and-done” method as it allowed several harvests and increased productivity (Johnson et al., 2016). Lettuce and some other leafy greens harvested by cut-and-come again method was tried on ISS and showed higher yield, although more comparisons to ground data are required to further improve the technique (Anderson et al., 2019).

CHAPTER 2. SELECTION OF OPTIMUM CONTROLLED-RELEASE-FERTILIZER COMBINATION FOR GROWING *LACTUCA SATIVA*'OUTREDGEOU UNDER ISS ENVIRONMENTAL CONDITIONS

2.1 Introduction

Food security for astronauts in long-term missions is a major challenge. Long-term storage leads to several unfavorable changes and significant decrease in nutrient content of packaged food compared to fresh produce (Zwart et al., 2009; Cooper et al., 2017). Furthermore, space flight, especially long duration space flight, results in oxidative damage, bone loss, and compromised vitamin D in astronaut's bodies (Smith et al., 2005). These problems could be avoided by producing fresh vegetables on-board to improve astronauts' diets, performance, and morale (Massa et al., 2017). Selection of appropriate crops and optimizing growing conditions to maximize yield and optimize mineral content is crucial for space farming (Hoff et al., 1982, Massa et al., 2015).

Lettuce has several characteristics essential for space agriculture such as high productivity, tolerance to osmotic stress, compact size, and tolerance of low light intensity. Hence, Lettuce as well as some other crops such as Chinese cabbage, cabbage, cauliflower, kale, turnip, Swiss chard, endive, dandelion, radish, New Zealand spinach, tampala, and sweet potato were suggested in the first published list of potential crops for space agriculture (Boeing Comp., 1962; Gouleke and Oswald, 1964). Moreover, Massa et al. (2015) in a down-selection experiment to recommend finalist candidate crops for space agriculture showed that 'Outredgeous' lettuce had good nutrition, good growth, and good palatability. Lettuce contains several healthy bioactive compounds and several essential dietary minerals such as magnesium (Mg), manganese (Mn), potassium (K), iron (Fe), zinc (Zn), calcium (Ca), and phosphorus (P) (Kim et al., 2016). Carotenoids (i.e. β-carotene and lutein) were reported in high quantities in several lettuces such as crisphead, butterhead, romaine, and green and red leaf lettuces (Mou, 2005; Nicolle et al., 2004).

Optimizing growing conditions to attain high yields and avoid several challenges related to space agriculture has several important pillars. Light-emitting diodes (LEDs) proved to be the most effective technology in controlled-environment crop production. Manipulating light spectral ratios affected lettuce growth and development (Yorio et al., 2001; Kim et al., 2004). The cut-and-come-

again harvest method significantly increased lettuce productivity (Johnson et al., 2016). Arcillite of grain size 1-2 mm in diameter appeared to be the best nutrient-delivery system for space farming, as it allows sufficient water and nutrient transfer to the root system with minimal problems (Monje et al., 2003).

Slow-release fertilizer (SRF) and controlled-release fertilizer (CRF) enhanced nutrient composition of the growing substrate (Handreck and Black, 2002). Temperature but not substrate type, texture, and pH affected release rate from controlled-release fertilizers (Broschat, 2005; Kochba et al., 1990; Oertli and Lunt, 1962; Shibata et al., 1980). At temperatures of 20°C to 30°C, Nutricote (18-6-8) fertilizers (T100, T270, and T360) showed the highest steady-state release rates (Adams et al., 2013).

The objective of this experiment is to identify optimal controlled-release fertilizer composition for growing lettuce plants under cultural and environmental conditions similar to the international space station.

2.2 Materials and Methods

2.2.1 Growth chamber and growing conditions:

This experiment was conducted in a walk-in growth chamber (EGC, Chagrin Falls, OH) mimicking ISS environmental conditions, except for microgravity. During the experiment, environmental conditions set to mimic the average conditions during the first Veggie flight aboard ISS, which were 24/22°C (D/N), 45/50% RH, continuous CO_2 level of 2,800µmolmol^{-1} , and photoperiod of 16/8 hours (Massa et al., 2016).

2.2.2 Biomass production system for education (BPSe)

BPSes are ground-based Veggie analogues consisting of a light-emitting diode (LED) cap in the top and reservoir in the bottom (Lee et al., 2004). Each BPSe was adjustable in height at the sides, which allowed maintaining constant distance of plants from the light cap throughout the experiment. Moreover, each BPSe had transparent accordion-action sides, which can be opened or closed throughout an experiment. Polyethylene curtains were installed between BPSes with the

white part facing inward to prevent light pollution between different treatments, and reduce light gradients within a treatment (Figures 2&3). Plants were grown in 4" square plastic pots. Each pot had a hole in each corner of the bottom which a 3" x 1" wick was installed. In addition, a 4" x 4" wick pad was installed below each pot to enhance water uptake and prevent substrate leakage (Figure 4). Plants received water from the reservoir by capillary wicking action. Each reservoir was a15" x 24" x 4" black plastic tray lined with a 2"-thick, open-cell foam sheet (Uline, WI) that was covered with wicking material (CapMat II, Phytotronics, Earth City, MO). Each light cap included three different light spectra including, blue (440-460 nm) with peak emission at 447.5 nm, green (520-550 at 530 nm), and red (620-645 nm) at 627 nm peak wavelengths (Figure 2).

2.2.3 Plant material and substrate:

Red Romaine Lettuce (*Lactuca sativa* cv. Outredgeous) was grown for 56 days in pots filled with 400 ml Profile Greens Grade Arcillite clay (Profile Products, Buffalo Grove, IL).

2.2.4 Treatments:

In this experiment, we incorporated a total of15g fertilizer/liter media, including 6 grams of 18-6-8 Nutricote controlled-release fertilizer in each pot filled with 400 ml substrate. Treatments were1) [50% (3 g) T70: 50% (3 g) T100], or2) [50 %(3 g) T70: 50 %(3 g) T180]. The T letter represents the time it takes to release 85% of the total nutrients when hydrate and under room temperature. For instance, T- 100 means it takes 100 days for this fertilizer to release 85% of its nutrients.

2.2.5 Experimental set up:

Our experimental design was a one-factor block design. All pots in the same tray had the same fertilizer treatment. Each BPSe included six pots setting on top of the mat-covered foam sheet. Plants received water from the reservoir by wicking capillary action, and water level was monitored from the tray's corner. Water level was maintained at half of the tray's height (almost 2"). Before starting the experiment, all reservoirs were set at the same position and distance from the light cap, so plants grown indifferent BPSes received the same light quality and quantity. All trays set all the way to the right side of the BPSes, and at three inches away from the front part of the light cap. The distance of the reservoir from the light cap set to 25 cm Using a Spectrometer

(Black-Comet, stellarNet Inc. Tampa Florid) light intensity was adjusted to provide total intensity of 330µmol/m2/s at the center point, with different spectral ratios of red, blue, and green light. It was set to provide 82% (270±2% µmol/m2/s) of the total intensity as red light, 9% (30±2% µmol/m2/s) as blue light, and 9% (30±2% µmol/m2/s) as green light. While performing light mapping, the light sensor was kept at the center point of the reservoir. The distance between light sensor and the BPSe's light cap maintained at 25 cm After performing light mapping and setting up all pots, this distance readjusted to 25 cm from BPSe's light cap, so plants grown in pots received the same intensity as what it was set for.

After finishing the light mapping, two seeds were sown in each pot, all reservoirs were watered with deionized water (DI) of pH 7 and EC zero. All reservoirs were watered until the sponge was fully hydrated and the water level was at half of the tray's height. During the germination stage (3-4 days from sowing seeds), all BPSe's accordion sides were closed to maintain high humidity during germination and seedling emergence. Ten days after planting, plants were thinned to one per pot. During the experiment as plants grew, the distance between canopy and light cap was readjusted to 25 cm as needed. Canopy center point was used as reference for height adjustment.

2.2.6 Data collection:

In this experiment, all plants were grown for 56 days and harvested three times throughout the experiment. The first harvest was at 28 days after planting; the second harvest was at 48 days after planting; and the final harvest was at 56 days after planting. During the first and second harvest, medium and large leaves were harvested and small leaves kept for re-growth. Small leaves were defined as any leaf equal to or less than 10cm length (Figure 5). During the first and second harvests, fresh weight, leaf area, leaf number, and dry weight were measured for large and medium leaves. All plant tissues harvested were placed in a drying oven at 60 °C for 2-3 days based on sample size. Once all plant tissues were completely dried and after measuring dry weight, all mid veins were removed and plant tissues from the same harvest and tray were ground together with a mortar and pestle to the finest size. Ground samples were sent to Great Lakes Labs (Fort Wayne, IN) for tissue mineral content analysis. After each harvest, the light cap height was re-adjusted to 25 cm

2.2.7 Leachate samples collection:

In addition, after cutting off the whole shoot, substrate leachate samples were collected following the pour-through method (LeBude, and Bilderback, 2009). 200 ml DI water was added to each pot, and pots in the same tray were drained together for 20-25 minutes, then pH and EC of the leachate combined samples were measured using HI 9813-6 portable pH & EC meter (pH/EC/TDS/C portable meter, Hanna Instruments Grocheck, Woonsocket, RI).

2.2.8 Statistical analysis:

The data were subjected to analysis of variance (ANOVA) using R software 3.4.3 (2017-11-30), within the R statistical package (R Foundation, Vienna, Austria) and PROC GLM SAS v.9.4 (SAS Institute, Cary, NC). When appropriate, means were separated by Tukey's HSD test at $P < 0.05$.

2.3 Results

2.3.1 Growth parameters for lettuce plants grown under two different controlled-release fertilizer treatments

Differences among harvests*:* Plants grown in 50% T100: 50% T70 showed a significant increase in shoot fresh weight from first to second harvest. However, no changes were observed from second to third harvest (Table1). Shoot fresh weight showed a 2.8-fold increases at the second harvest compared to the first harvest (Table1). Shoot dry weight increased significantly from harvest to harvest, as shoot dry weight increased by almost 2.3 fold and 1.2 fold at second and third harvests, respectively. Leaf area showed similar results to shoot fresh weight. However, leaf number followed the same trend as shoot dry weight. During the second harvest, leaf area increased by almost 2.5 fold. However, leaf number increased by 1.4 and 1.5 fold at second and third harvests, respectively (Table 1).

Plants grown in 50% T180: 50% T70 showed significant increase in the second harvest compared to the first harvest in shoot fresh weight, shoot dry weight, and leaf area, by 2, 2.7 and 2.5 fold, respectively. On the other hand, leaf number increased significantly from harvest to harvest, with 35 % and 28 % increases in leaf number detected at second and third harvests, respectively (Table 1).

Effect of treatments: Non-significant trends occurred between treatments in shoot fresh weight, leaf area, and leaf number. However, shoot dry weight was significantly higher for plants grown in 50% T100: 50% T70 compared to those grown in 50% T180:50% T70 (Table 1). In our investigation, during the first and second harvests, we did not encounter any treatment effect on fresh weight, dry weight, leaf area, or leaf number (Table 2).However, during the third harvest, 50% T180:50% T70 fertilizer treatment led to significant decrease ($P<0.05$) in fresh weight, dry weight, leaf number, and ($P=0.07$) in leaf area for the third harvest (Table 2).

2.3.2 Tissue mineral analysis for lettuce plants grown under two different controlled-release fertilizer treatments

Differences among harvests for plants grown in 50% T100: 50% T70 fertilizer treatment:

Both nitrogen and potassium decreased significantly at the third harvest compared to the first and second harvests (Table 3). However, sulfur calcium, iron, and aluminum did not change significantly from harvest to harvest (Table 3). Magnesium increased significantly from harvest to harvest (Table 3). Potassium decreased significantly by the third harvest and did not show significant differences between first and second harvests (Table 3). In contrast, sodium and manganese increased significantly by the third harvest and did not show significant differences between first and second harvest. (Table 3). Boron and zinc increased significantly from the first to the second harvest and did not show significant differences between second and third harvests (Table 3).

Differences among harvests for plants grown with 50% T180: 50% T70 fertilizer treatment:

Nitrogen tissue content decreased significantly from harvest to harvest, and reached its lowest value of 3.64% by the third harvest. Sulfur and phosphorus decreased significantly at the second and third harvests compared to the first harvest. Potassium decreased significantly at the third harvest compared to the first and second harvests. Magnesium, sodium, and manganese increased significantly at the third harvest compared to both the first and the second harvest. Calcium decreased significantly from first to second harvest. However, boron and zinc increased significantly at the second harvest. Copper decreased significantly from first to second harvest, although by the third harvest cupper was not significantly different from what it was at either the

first or the second harvest. Finally, iron and aluminum did not indicate any significant differences among all three harvests (Table 4).

Effect of fertilizer treatments on mineral contents within each harvest:

In the first harvest, lettuce plants treated with 50%T180:50%T70 showed significant increase in potassium, calcium, zinc and magnesium compared to lettuce plants treated with 50%T100:50%T70. In contrast, in the second harvest, lettuce plants treated with 50%T180:50%T70 showed a significant decrease in nitrogen content compared to lettuce plants treated with 50%T100:50%T70. Moreover, at the final harvest we did not find significant differences in tissue minerals content between the two fertilizer treatments (Table 5).

2.3.3 Substrate leachate analysis

We did not find significant differences in pH or EC between the two fertilizer treatments. All (Table 5).

2.4 Discussion

In this experiment, we investigated the release pattern from two different treatments of PCF Nutricote type 18-6-8. Mineral content is dependent on growing conditions such as temperature (Karmas and Harris1988), fertilizer (Ducsay and Varga 2003; Premuzic et al. 2004), irrigation (Pan-chal et al. 2001; Radovich et al. 2005), cultivation methods (Worthington 2001), and cultivar (Petříková and Pokluda 2003; Ghe-bramlak et al. 2004).

Our data showed that the content of some minerals decreased and others increased significantly from harvest to harvest. However, mineral contents were within USDA recommended ranges for human consumption (USDA, 2019).The release period from PCF prills is divided into three different stages; first stage is lag phase where release rate is very slow; second stage is a linear phase where release rate increases; and last stage is decay phase where release rate declines (Shavivet al., 2003). In our investigation, during the first and second harvests 28 and 42 days after planting, we did not encounter any treatment effect on fresh weight, dry weight, leaf area, or leaf number (Table 2). We assume that both T100 and T180 were in the lag phase at that time, and T70

was the main nutrient resource. Moreover, P and K were significantly higher in the T100 treatment compared to the T180 treatment, but this did not influence our growth parameters (Table 2).High concentrations of P and K did not cause significant increase in yield when N concentration was low (Hoque et al.,2010).

At the second harvest, T180 should have started to enter the linear phase. However, T100 should have entered that phase earlier, so more nutrients were released from T100 than T180. Our data support this hypothesis as during the second harvest shoot fresh weight was 17% higher in the fertilizer treatment incorporating 50% T100 treatment compared to 50% T180 (P=0.07) (Table 2).

By the third harvest, both fertilizers (T100 and T180) should have been in the same release phase, so we did not find significant differences in tissue mineral content between the two fertilizer treatments (Table 5). However, fresh weight, dry weight, and leaf number were significantly higher in the 50% T100 treatment compared to the 50% T180 treatment (Table 2). These differences most likely were due to high rates of carbon assimilation in the 50%T100 treatment. After the second harvest, at least 3-5 small leaves remained that would grow out by the third harvest. A significant part of the harvested biomass in the third harvest was from those small leaves that remained after the second harvest. As mentioned previously, at the second harvest N tissue content was significantly higher in the 50%T100 treatment, which may have supported higher growth in that treatment. Increased carbon assimilation corresponded with a significant increase in leaf tissue content of N (Field and Mooney, 1986; Evans, 1989). Carbon assimilation depends on N content in leaf tissues (Field and Mooney, 1986; Evans, 1989). At the second harvest, N tissue content was significantly higher in plants grown in [50%T100: 50%T70] (Table 5), and shoot dry weight was higher in the 50% T100 treatment (P=0.08) (Table 2). The significant increase in N corresponded within increased shoot dry weight with almost the same percentage (nitrogen increased by 13% and shoot dry weight increased by 14%). Total yield from the three harvests indicated that shoot dry weight was significantly higher in lettuce plants grown with a fertilizer treatment of [50%T100: 50%T70] compared to those grown with [50%T180:50%T70].

In conclusion, [50%T100: 50%T70] and [50%T180: 50%T70] showed different effects on tissue mineral content and growth parameters of lettuce. Moreover, [50%T100: 50%T70] increased

lettuce growth parameters in the second and third harvest, and increased total shoot dry weight compared to [50%T180: 50%T70]. Hence, under the cultural and environmental conditions of ISS we recommend fertilizer treatment of [50%T100: 50%T70] for growing Red Romanian lettuce. However, substrate leachate analysis indicated very high EC in both fertilizer treatments (Table 6). In both treatments EC was higher than half strength hydroponic solution and might be a growth limiting. Therefore testing lower fertilizer ratio might be beneficial in identifying optimal fertilizer treatment.

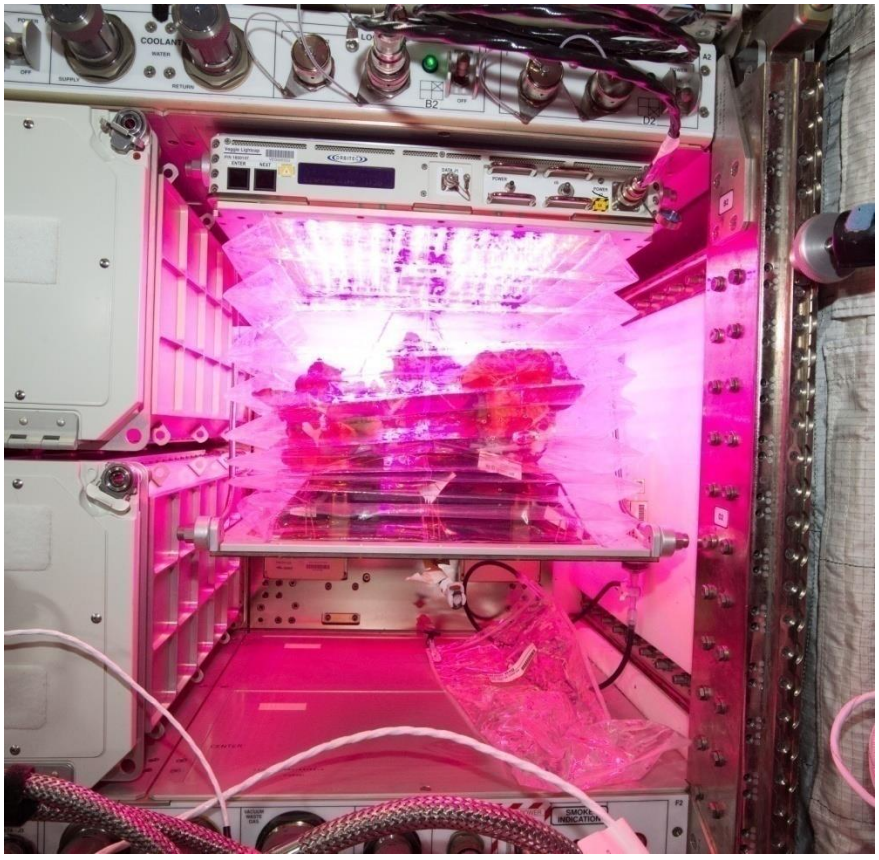

Figure 1 VEGGIEE, vegetable production system on the international space station.

Figure 2 Biomass production system for education.

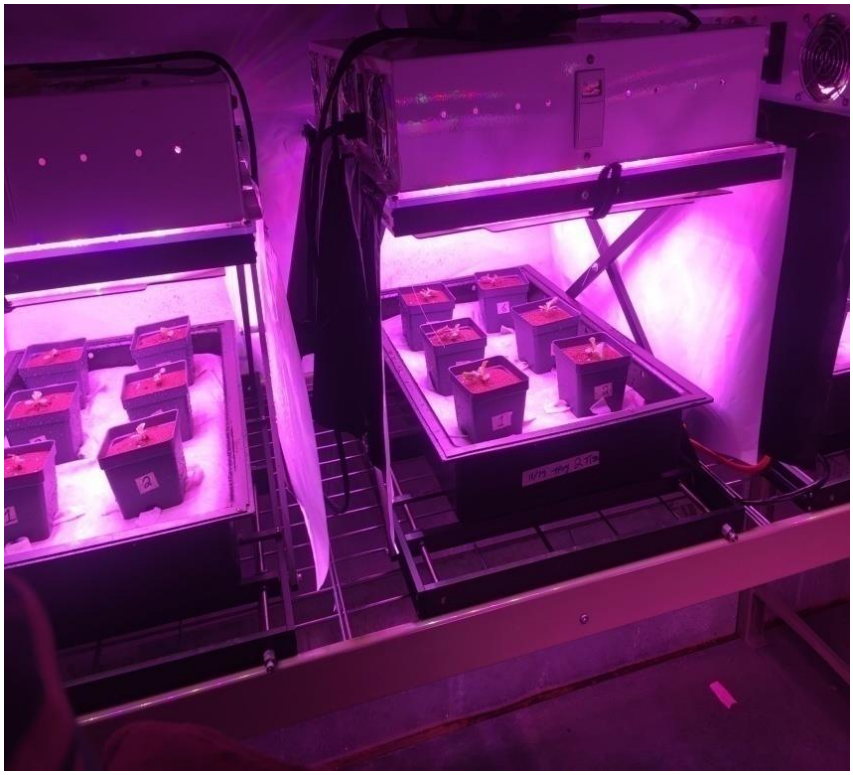

Figure 3 Experimental set up at Purdue, each BPSe had six pots setting in top of covered foam sheet.

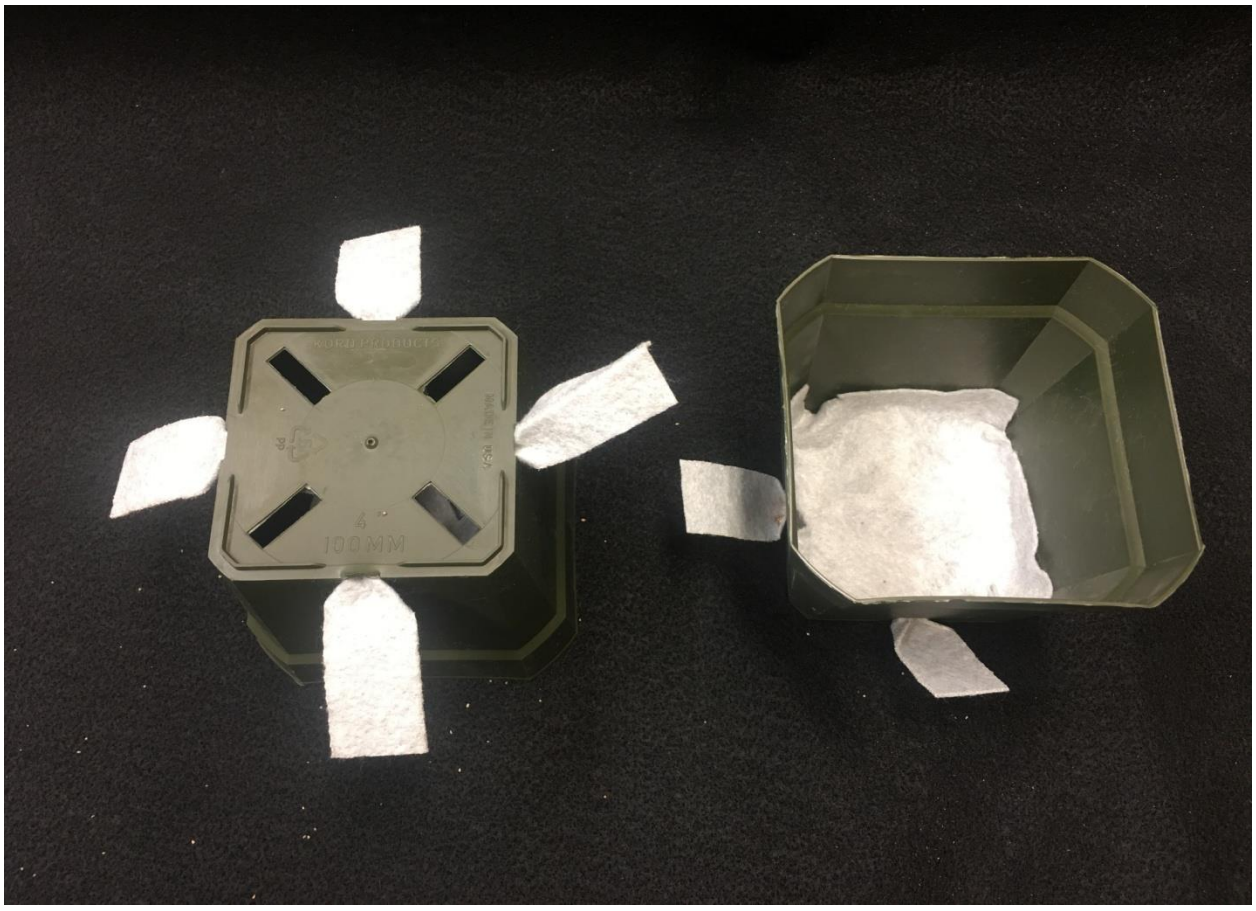

Figure 4 Wicks set up on 4” square pots

Table 1 Effect of fertilizer formulation and harvest time on total yield (fresh weight, dry weight, leaf area, and leaf number) of lettuce plants grown under two different fertilizer treatments: 50%T100:50%T70, and 50%T180:50%T70. Each value is the average of five replicates.

Harvest	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Leaf number
		50%T100:50%T70		
Harvest 1	17.15 b[z]	1.39 c	569.18 b	8.18 c
Harvest 2	47.74 a	3.42 b	1421.67 b	11.59 b
Harvest 3	52.33 a	4.09 a	1470.99 a	17.02 a
Total/Plant	118.48 A[y]	8.83 A	3454.12 A	37.09 A
		50%T180:50%T70		
Harvest 1	20.53 b	1.13 b	497.09 b	7.42 c
Harvest 2	40.44 a	3.04 a	1249.25 a	11.44 b
Harvest 3	40.29 a	3.19 a	1272.54 a	15.92 a
Total/Plant	105.38 A	7.48 B	3458.45 A	34.57 A

[z]Mean separation among harvests within the same fertilizer treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y] Effect of fertilizer treatment on yield ($P< 0.05$), whereby means associated with different letters are significantly different

Table 2 Effect of fertilizer on yield (fresh weight, dry weight, leaf area and leaf number). Each value is the average of five replicates.

Fertilizer	**Fresh weight**	**Dry weight**	**Leaf area**	**Leaf Number**
	(g)	**(g)**	**(cm^2)**	**(Leaves)**
First harvest				
50%T100:50%T70	17.15	1.39	569.18	8.18
50%T180:50%T70	20.53	1.13	497.09	7.42
Significance (*P*<0.05)[z]	0.64	0.12	0.29	0.18
Second harvest				
50%T100:50%T70	47.74	3.42	1421.67	11.59
50%T180:50%T70	40.44	3.04	1249.25	11.44
Significance (*P*<0.05)	0.07	0.08	0.21	0.77
Third harvest				
50%T100:50%T70	52.33	4.09	1470.99	17.02
50%T180:50%T70	40.29	3.19	1272.54	15.92
Significance (*P*<0.05)	**	*	0.07	*

[z]Mean separation among harvest by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

Table 3 Effect of harvest on tissue mineral content. Plants grew in100% profile substrate and 50%T100:50%T70 controlled-release fertilizer. Each value is the average of five replicates.

Mineral		**Harvest1**		**Harvest2**		**Harvest3**	
N[y]	**(%)**	5.56	a[z]	5.27	a	3.85	b
S	**(%)**	0.35	a	0.34	a	0.29	a
P	**(%)**	0.50	a	0.47	ab	0.36	b
K	**(%)**	6.49	a	6.63	a	4.36	b
Mg	**(%)**	0.28	c	0.34	b	0.40	a
Ca	**(%)**	0.39	a	0.35	a	0.33	a
Na	**(%)**	0.10	b	0.13	b	0.18	a
B	**(ppm)**	50.67	b	69.50	a	72.83	a
Zn	**(ppm)**	23.67	b	40.33	a	45.17	a
Mn	**(ppm)**	673.67	b	719.00	b	897.33	a
Fe	**(ppm)**	99.33	a	114.67	a	105.83	a
Cu	**(ppm)**	7.00	a	6.00	ab	5.33	b
Al	**(ppm)**	39.33	a	23.50	a	18.67	a

[z]Mean separation among harvests by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum

Table 4 Effect of harvest on tissue mineral content. Plants grew in 100% profile substrate and 50%T180:50%T70 controlled release fertilizer. Each value is the average of five replicates.

		Harvest1		**Harvest2**		**Harvest3**	
N[y]	**(%)**	5.51	a[z]	4.61	b	3.64	c
S	**(%)**	0.38	a	0.32	b	0.31	b
P	**(%)**	0.51	a	0.41	b	0.34	b
K	**(%)**	6.82	a	5.89	a	4.60	b
Mg	**(%)**	0.29	b	0.32	b	0.44	a
Ca	**(%)**	0.43	a	0.33	b	0.37	b
Na	**(%)**	0.11	b	0.11	b	0.18	a
B	**(ppm)**	57.33	b	66.17	ab	80.83	a
Zn	**(ppm)**	28.33	b	40.00	ab	48.00	a
Mn	**(ppm)**	754.33	b	706.50	b	1037.83	a
Fe	**(ppm)**	103.67	a	97.83	a	115.67	a
Cu	**(ppm)**	6.67	a	5.17	b	6.17	ab
Al	**(ppm)**	13.67	a	24.33	a	19.17	a

[z]Mean separation among harvest by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum.

Table 5 Effect of fertilizer on tissue mineral content. Each value is the average of five replicates.

Fertilizer	N[x] (%)	S (%)	P (%)	K (%)	Mg (%)	Ca (%)	Na (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Al (ppm)
First harvest													
50%T100:50%T70	5.6	0.35	0.50	6.5	0.28	0.39	0.10	50.7	23.7	673.7	99.3	7.0	39.3
50%T180:50%T70	5.5	0.38	0.51	6.8	0.29	0.43	0.11	57.3	28.3	754.3	103.7	6.7	13.7
Significance (*P*<0.05)[z]	NS	NS[y]	NS	*	NS	*	NS	NS	*	*	NS	NS	NS
Second harvest													
50%T100:50%T70	5.3	0.34	0.47	6.6	0.34	0.35	0.13	69.5	40.3	719.0	114.7	6.0	23.5
50%T180:50%T70	4.6	0.32	0.41	5.9	0.32	0.33	0.11	66.2	40.0	706.5	97.8	5.2	24.3
Significance (*P*<0.05)	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Third harvest													
50%T100:50%T70	3.8	0.29	0.36	4.4	0.40	0.33	0.18	72.8	45.2	897.3	105.8	5.3	18.7
50%T180:50%T70	3.6	0.31	0.34	4.6	0.44	0.37	0.18	80.8	48.0	1037.8	115.7	6.2	19.2
Significance (*P*<0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Mean separation among treatments by Tukey HSD (*P*< 0.05), whereby means associated with different letters are significantly different

[y]NS, non-significant

[x](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum

Table 6 pH and EC of substrate leachate. Samples collected from substrate containing residual fertilizer after the final harvest. Each value is the average of three replicates.

Fertilizer	**pH**	**EC**
50%T100:50%T70	**5.67**	**1.54**
50%T180:50%T70	**6.05**	**1.32**
Significance (*P*<0.05)	**NS**	**NS**

[z]Mean separation among treatments by Tukey HSD ($P < 0.05$), whereby means associated with different letters are significantly different
[y]NS, non-significant

CHAPTER 3. SELECTION OF OPTIMUM CONTROLLED-RELEASEFERTILIZER, AS WELL AS SUBSTRATE COMBINATION FOR GROWING *BRASSICA RAPA* MIZUNA UNDER ISS ENVIRONMENTAL CONDITIONS

3.1 Introduction

Long-term crewed space missions to outer space necessitate special consideration for astronauts' diets. Forshort-term missions to low Earth orbit, food can be delivered to astronauts via re-supply trips. Astronaut bodies have shownoxidative damage, bone loss, and compromised vitamin D during space flight (Smith et al., 2005). As well, packaged food loses a significant amount of nutrientsduring long-term storage (Zwart et al., 2009). The National Aeronautics and Space Administration (NASA) has tested several production systems to produce fresh vegetables on board the International Space Station (ISS). The most recent system in current use is a small growth chamber called Veggie (Massa et al., 2017) (Figure 1). Optimizing growing conditions and testing candidate plants requiresground studies to obtain baselineyield and mineral content to compare with crop performance on-orbit (Massa et al., 2015).

Most Brassica cultivars are characterized as important dietary sources for calcium and iron. For example, 200 g Brassica daily will suffice human need foriron (Artemyeva and Solovyeva, 2006). In addition, Brassica are well known for their high carbohydrates, protein, water content, fiber, and secondary metabolites, and they protect human bodies from cardiovascular diseases (King and Barker 2003). Mizuna, *Brassica rapa* var. japonica, is a Japanese cultivar that is increasing in importance. In a recent down-selection experiment for candidate plant species to be grown in space,'Mizuna' showed excellent growth and was fairly palatable and nutritious underground-basedgrowing conditions similar to those on ISS. Thus, Mizuna is an promising candidate for cut-and-come-again harvest scenarios on ISS.

Determining optimum combinations of substrate, fertilizer, and light quality plays a key role in optimizing yield and mineral content of growing plants. Several recent reports showed light-emitting diodes (LEDs) as the most successful light source for space agriculture (Massa et al., 2013, 2016). LEDs provide an easy way to manipulate spectral ratios (Nelson and Bugbee, 2014;

2015) while growing crops in small space (Poulet et al., 2014), and to avoid heat-related stress. Substrate choice to grow plants in the space environment should consider several important features such as small available space (Massa et al., 2013), avoidingcontamination (Massa et al., 2013; Stutte et al., 2011), and allowingsufficient water and nutrient transfer to the root zone (Monje et al., 2003). Solid substrates currently are the preferable choice for long space missions as they can made mechanically reliable and allow sufficient water and nutrient transfer to the root zone (Monje et al., 2003). Arcilite manufactured from calcined clay has shown uniform results when tested with three different lettuce cultivars (Flemming et al., 2012). Solid fertilizers are the nutrition resource of choice to grow plants in inorganic media in microgravity. Both controlled-release fertilizer (CRF) and slow- release fertilizers (SRF) improved nutrient content in solid substrates (Handreck and Black, 2002). Nutrient-release rates of three different CRFs were not impacted by water, moisture, and solid substrate status (Adams et al., 2013). Furthermore, nutrient release byNutricote (18-6-8) fertilizers (T100, T270 and T-360) showed the highest delivery steady-state release rate within a temperature range of 20°C to 30°C (Adams et al., 2013). Nutrient-release rate is affected by neither substrate type, texture, pH, nor biological activity (Broschat, 2005; Kochba et al., 1990; Oertli and Lunt, 1962; Shibata et al., 1980).

In the current study, we investigated the effect of two different fertilizers [50% T70: 50% T100] and [50 %T70: 50 % T180], and two different substrates, 100% Profile and 40% Profile: 60% Turface on growth parameters and mineral content of Mizuna, *Brassicarapa* var. japonica, under cultural and environmental conditions similar to ISS. We hypothesized that using a mix of fine and coarse substrate particles would improve the physical properties of the substrate and increase the yield of Mizuna plants.

3.2 Materials and Methods

3.2.1 Growth chamber and growing conditions

This experiment was conducted in a walk-in growth chamber (EGC, Chagrin Falls, OH) mimicking ISS environmental conditions, except for microgravity. During the experiment, environmental conditions were set to mimic average conditions during the first Veggie flight

aboard ISS, which were 24/22°C (D/N), 45/50% RH (D/N), a continuous CO_2 level of 2,800µmolmol^{-1} , and photoperiod of 16/8 hours (Massa et al., 2016).

3.2.2 Biomass production system for education (BPSe)

BPSes are ground-based Veggie analogues consisting of a light-emitting diode (LED) cap in the top and a reservoir in the bottom (Lee et al., 2004). Each BPSe was adjustable in height at the sides, which allowed height readjustments for maintaining constant distance of plants from the light cap throughout the experiment. Moreover, each BPSe had transparent accordion-action sides, which can be opened or closed throughout an experiment. Polyethylene curtains were installed between BPSes with the white part facing inward to prevent light pollution between different treatments, and reduce light gradients within a treatment (Figures 2&3). Plants were grown in 4" square plastic pots. Each pot had a hole in each corner of the bottom through which a 3" x 1" wick was installed. In addition, a 4" x 4" wick pad was installed below each pot to enhance water uptake and prevent substrate leakage (Figure 4). Plants received water from the reservoir by capillary-wicking action. Each reservoir was a15" x 24" x 4" black plastic tray lined with a 2"-thick, open-cell foam sheet (Uline, WI) that was covered with wicking material (CapMat II, Phytotronics, Earth City, MO). Each light cap included three different LEDs including blue (440-460 nm) with peak emission at 447.5 nm, green (520-550)at 530 nmpeak wavelengths, and red (620-645 nm) at 627 nm peak wavelengths, each dimmable from 100% to 0%for spectralblending.

3.2.3 Substrate analysis

Before starting the experiment we wanted to identify the optimum substrate composition in terms of substrate physical properties. So we started bytesting physical properties (Container capacity, air space, total porosity, and bulk density) of four different substrate compositions; 100% Turface, 50% Turface: 50% profile, 30% Turface: 70% profile, and 10% Turface: 90%profile, following North Carolina State University protocol (Fontenon&Harden, 2003). Based on astandard curve established by our four treatments, we found that, in order to meet the standard substrate physical properties listed by (Choi et al., 2019); our substrate should include the ratio of 40% Profile: 60% Turface (Table 7).

3.2.4 Plant material and substrate treatments

Mizuna (*Brassica rapa* var. japonica) was grown for 56 days in pots filled with either 400 ml Profile Greens Grade Arcillite clay (Profile Products, Buffalo Grove, IL), or 160ml Profile mixed with 240 ml Turface.

3.2.5 Fertilizer treatments

In this experiment, we incorporated a total of15g fertilizer/liter media, equating 6 grams of 18-6-8 Nutricote controlled-release fertilizer in each pot filled with either 400 ml profile or 160 ml Profile mixed with 240 ml Turface.

Two different fertilizer treatments were applied to each substrate treatment. Our treatments were1) [50% (3 g) T70: 50% (3 g) T100] & 100% profile substrate, 2) [50 %(3 g) T70: 50 %(3 g) T180]& 100% profile substrate, 3) [50% (3 g) T70: 50% (3 g) T100] & 40% profile: 60% Turface substrate and 4) [50 %(3 g) T70: 50 %(3 g) T180] & 40% profile: 60% Turfacesubstrate. The T letter represents the time it takes to release 85% of the total nutrients. For instance, T- 100 means it takes 100 days for this fertilizer to release 85% of its nutrients.

3.2.6 Experimental set up

Our experimental design was randomized block design. All pots in the same tray received same fertilizer and substrate treatment. Each BPSe included six pots setting on top of the mat-covered foam sheet. Plants received water from the reservoir by wicking capillary action, and water level was monitored from the tray's corner. Water level was maintained at half tray height (almost 5 cm). Before starting the experiment, all light caps were set at the same position and distance from the reservoirs, so plants grown indifferent BPSes received the same light quality and quantity. All trays were set all the way to the right side of the BPSes, and 7 cminfrom the front part of the light cap. The distance betweenthe reservoir and light cap was maintained at 25 cm.usingaSpectrometer (Black-Comet, stellarNet Inc. Tampa Florid). Light intensity was adjusted to provide a total PPFDof 330µmol/m^2/s at the center point, It was set to provide 82% (270±2% µmol/m^2/s) of the total intensity as red light, 9% (30±2% µmol/m^2/s) as blue light, and 9% (30±2% µmol/m^2/s) as green light. While performing light mapping, the light sensor was kept at the center point of the

reservoir. The distance between the light sensor and the BPSe's light cap was maintained at 25 cm. After performing light mapping and setting up all pots, this distance was readjusted to 25 cm from BPSe's light cap, so plants grown in pots received the same intensity as what was originally set.

After finishing the light mapping, two seeds were sown in each pot; all reservoirs received deionized water (DI) of pH 7 and EC zero. All reservoirs were watered until the sponge was fully hydrated and the water level was at half of the tray's height. During the germination stage (3-4 days from sowing seeds), all BPSe's accordion sides were closed to maintain high humidity during germination and seedling emergence. Ten days after planting, plants were thinned to one per pot. During the experiment as plants grew, the distance between canopy and light cap was readjusted to 25 cm as needed. Canopy center point was used as reference for height adjustment.

3.2.7 Data collection

In this experiment, all plants were grown for 56 days and harvested three times throughout the experiment. The first harvest was at 28 days after planting; the second harvest was at 42 days after planting; and the final harvest was at 56 days after planting. During the first and second harvest, medium and large leaves were harvested and small leaves kept for re-growth. Small leaves were defined as any leaf equal to or less than 10cm length. During the first and second harvests, fresh weight, leaf area, leaf number, and dry weight were measured for large and medium leaves. All plant tissues harvested were placed in a drying oven at 60 °C for 2-3 days based on sample size. Once all plant tissues were completely dried and after measuring dry weight, all mid veins were removed and plant tissues from the same harvest and tray were ground together with a mortar and pestle to the finest size. Ground samples were sent to Great Lakes Labs (Fort Wayne, IN) for tissue mineral content analysis. After each harvest, the light cap height was re-adjusted to 25 cm

3.2.8 Leachate samples collection and analysis

In addition,after the final harvest and after cutting off the whole shoot, substrate leachate samples were collected following the pour-through method (LeBude, and Bilderback, 2009). 200 ml DI water was added to each pot, and pots in the same tray were drained together for 20-25 minutes,

then pH and EC of the leachate combined samples were measured using anHI 9813-6 portable pH & EC meter (pH/EC/TDS/C portable meter, Hanna Instruments Grocheck, Woonsocket, RI).

3.2.9 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using R software 3.4.3 (2017-11-30) within the R statistical package (R Foundation, Vienna, Austria) and PROC GLM SAS v.9.4 (SAS Institute, Cary, NC). When appropriate, means were separated by Tukey's HSD test at $P < 0.05$.

3.3 Results

3.3.1 Growth parameters

a- Mizuna plants grown in 100 % profile and fertilizer treatment of 50%T100:50%T70.

Shoot fresh weight and dry weight showed significant increasesatthe second harvest compared to the first harvest by 52% and 38%, respectively. In contrast, non-significant differences occurred atthe third harvest compared to the second harvest (Table 8). Leaf area and leaf number showed different trends. Leaf area did not change significantly from first to second harvest, but increased significantly from first to third harvest. Leaf number increased significantly at both second and third harvests by 81% and 52%, respectively (Table 8).

b- Growth parameters for Mizuna plants grown under100 % profile and fertilizer treatment of 50% T180: 50% T70.

Mizuna plants grown under fertilizer treatment of 50% T180: 50% T70 did not show significant changes in shoot fresh weight, shoot dry weight, orleaf area from harvest to harvest. However, leaf number showed a significant increase at the second and third harvest compared to the first harvest (Table 8).

c- Growth parameters for Mizuna plants grown in asubstrate mix of 40 % Profile: 60% Turface and a fertilizer treatment of 50% T100: 50% T70.

Fresh weight, leaf area, and leaf number did not change significantly from harvest to harvest. However, shoot dry weight increased significantly at the third harvest compared to the first harvest only (Table7).

d- Growth parameters for Mizuna plants grown insubstrate mix of 40 % Profile: 60%Turface and a fertilizer treatment of 50%T180: 50% T70.

Shoot fresh weight showed significant reduction atthe second and third harvests compared to the first harvest (Table 8). However, shoot dry weight did not show significant differences among all harvests. Leaf area was significantly higher at the first harvest compared to the third harvest. On the other hand, leaf number was significantly lower at the second harvest compared to the third harvest (Table 8). However, leaf area was not significantly different at the second harvest from first and third harvests, and leaf number was not significantly different at the first harvest from second and third harvests (Table8).

3.3.2 Tissue mineral analysis

a- Mineral content of Mizuna plants grown in 100 % Profile and a fertilizer treatment of 50%T100:50%T70.

Phosphorus, magnesium, zinc, manganese, and iron did not show significant differences among all harvests. Nitrogen decreased significantly from harvest to harvest. Calcium and potassium were significantly lower at the third harvest compared to the first and second harvests (Table 9). In contrast, copper and aluminum were significantly higher at third harvest compared to first and second harvests (Table 9). Sulfur did not show significant differences at the first harvest; however, it decreased significantly from second to third harvest. Sodium and boron increased significantly at the second harvest. At the third harvest, sodium decreased significantly, however boron did not show significant changes (Table 9).

b- Mineral content of Mizuna plants grown in 100 % Profile and fertilizer treatment of 50% T180: 50% T70

Calcium, iron, and copper did not change significantly during all harvests. Nitrogen and potassium decreased significantly from harvest to harvest. Phosphorus, magnesium, and zinc were

significantly higher at third harvest compared to the first harvest only. Manganese and aluminum increased significantly from second to third harvest; however, they did not show significant differences between first and second harvest. Sulfur and boron decreased significantly from first to second harvest; at third harvest sulfur increased again, although boron decreased. Sodium did not show significant differences between first and second harvest; however, it decreased significantly during the third harvest (Table 10).

c- Mineral content of Mizuna plants grown in substrate mix of 40 % Profile: 60 % Turface and fertilizer treatment of 50%T100: 50% T70.

Calcium, copper, and aluminum did not change significantly from harvest to harvest. In contrast, nitrogen, potassium, and calcium decreased significantly from harvest to harvest, and magnesium increased significantly from harvest to harvest (Table 11). Sulfur decreased significantly at the second and third harvest compared to the first harvest. Sodium and boron decreased significantly at the third harvest compared to both the first and the second harvests (Table 11). Iron and magnesium increased at the third harvest compared to the first and the second harvests. Zinc increased at the third harvest compared to the first harvest only (Table 11).

d- Mineral content of Mizuna plants grown in a substrate mix of 40 % Profile: 60 % Turface and fertilizer treatment of 50% T180: 50% T70.

Phosphorus, calcium, sodium, boron, and zinc did not show any significant differences among all harvests (Table 12). Nitrogen, sulfur, and potassium were significantly higher at the first harvest compared to the other two harvests. Iron and aluminum were significantly higher at the third harvest compared to the first and the second harvests. Magnesium, manganese, and copper were significantly higher at the third harvest compared to the first harvest only.

3.3.3 Effect of treatment on growth parameter

Both substrate and fertilizer showed significant effects on fresh weight and dry weight. However, non-significant interaction existed between substrate and fertilizer (Table 13). 100% Profile substrate showed higher fresh weight and leaf area compared to 40% Profile, and 50%T100:50%T70 fertilizer showed higher leaf area and fresh weight compared to

50%T180:50%T70. Nevertheless, 40% Profile substrate and 50%T180:50%T70 fertilizer gave a significant reduction in fresh weight and leaf area compared to the other treatments except fresh weight 100% Profile with the same fertilizer treatment (50%T180:50%T70) (Table 13).

Treatments showed different patterns of effect on different growth parameters in each harvest, except for dry weight, as no significant differences existed among treatments in any harvest (Table 13). In addition, fresh weight and leaf area in the first harvest and leaf number in the third harvest did not show significant differences among treatments (Table 13).

3.3.4 Effect of treatments on mineral content

Substrate had a significant effect on sulfur, potassium, calcium, sodium, zinc, and aluminum. However, fertilizer had a significant effect on nitrogen, phosphorus, sodium, and manganese (Table 14). Moreover, we observed different patterns of effect within each harvest on different minerals, with the exception of magnesium and manganese,which did not show any significant effect of treatments within each harvest (Table 14).

3.3.5 Substrate leachate analysis

Our substrate leachate analysis did not show significant difference in pH or EC between all treatments; however, EC was high in all treatments (Table 15).

3.4 Discussion

Using substrate with appropriate particle size is critical for providing adequate water uptake and aeration. Large-grain soil allows appropriate aeration but limits water uptake, and small grains allow appropriate water uptake but may limit aeration (Casado 2006). Our experiment results suggested that under the cultural and environmental conditions of ISS, on the groundmizuna plants grow better in substrate treatment of 100 % profile. Since shoot fresh weight, leaf area, and leaf number in different harvests increased significantly when mizuna plants grow in substrate treatment of 100 % profile, compared with substrate treatment of 40% profile: 60% Turface(Table 8). Fertilizer also had significant effect on yield; we found a significant effect of fertilizer treatment of 50%T100:50%T70 on shoot fresh weight and leaf number (Table 8).

In 2013 Adams et al.,reported, nutrient-release rates from PCF Nutricote Total with Minor Nutrients (ChissoAsahi Fertilizer Company, LTD., Tokyo, Japan) werenot impacted by water, moisture, and solid substrate status. MoreoverBroschat, 2005; Kochba et al., 1990; Oertli and Lunt, 1962; Shibata et al., 1980 reported that nutrient-release rate were not impacted neither by substrate type nor texture. Our results supported those findings, as we did not find significant interactions between fertilizer and substrate (Table 8).

Growing mizuna plants in a substrate of small particles size, 100% Profile, affected all growth parameters, except for dry weight (Table 8). Apparently, small substrate particles allowed adequatewater uptake (Casado 2006), so plants grown in 100% profile had higher moisture content, which increased their fresh weight but did not affect dry weight.

In this investigation, mineral content, especially macronutrients, showed direct relationship with yield. For instance, at the third harvest, plants grown in 40 % Profile did not show significant differences in yield between both fertilizer treatments. This was correlated withthe tissue mineral analysis, as we did not find significant differences in tissue mineral content as well (Tables 13 and 14). On the other hand, at the third harvest plants grown in 100% Profile and 50% T100:50% T70 had the highest yield associated with the highest levels of macronutrients (N, P, and k). In contrast, plants grown in 40% Profile and fertilizer treatment of 50% T180:50% T70 had the lowest yield associated with the lowest macronutrients (Tables 13 and 14).

In terms of substrate, this experiment raised a big concern about using arcillite as all treatments showed high levels of manganese; this was similar to the findings of (Samuel et al., 2019). Based on the findings of Adams and others (2013), manganese levels of our treatments were way beyond optimum levels.

Substrate leachate analysis indicated normal levels of pH and high levels of EC in all treatments. However, no significant differences were found between treatments in pH, or EC (Table 15).
EC could be a limiting factor in plant growth and development. Previous research showed significant decreases in yield with increases in EC (Miceli et al., 2003; Serio et al., 2001; and

Samarakoon, 2006). In addition, high EC indicates that lots of nutrients were still available in the substrate, so using less fertilizer would be more efficient and might increase yield.

From this study we concluded that, mizuna plants grow better in a substrate treatment of 100% Profile compared with substrate treatment of 40%profile: 60% Turface. In addition growing Mizuna plants in fertilizertreatment of 50%T100:50%T70 increased shoot fresh weight and leaf number significantly. Moreover, release rate of controlled release –fertilizer, Nutricote, type 18-6-8 was not affected by substrate particles size, as our results did not show significant interaction between substrate and fertilizer. Finally, plants grown in 100% Profile and 50% T100:50%T70 had the highest yield associated with the highest levels of macronutrients (N, P, and K).

Table 7 Physical properties of four different substrate compositions. Each value is the average of three replicates.

Parameter	**100% Turface**	**50% Turface**	**30% Turface**	**10% Turface**
Container capacity	40.8	46.4	53.1	55.0
Air space	24.8	8.0	4.9	3.1
Porosity	65.5	54.5	58.0	58.1
Bulk density	0.541	0.478	0.478	0.465

Table 8 Effect of fertilizer formulation, substrate and harvest time on total yield (fresh weight, dry weight, leaf area, and leaf number) of mizuna plants grown under two different fertilizer treatments: 50%T100:50% T70 and 50% T180:50%T70; and two different substrate: 100% profile and 40% profile. Each value is the average of five replicates.

	Fresh weight (g)	**Dry weight (g)**	**Leaf area (cm^2)**	**Leaf Number**
		100% profile & 50%T100:50%T70		
Harvest 1	23.64 b[z]	2.05 b	501.84 b	18.1 c
Harvest 2	35.94 a	2.86 a	741.06 ab	32.72 b
Harvest 3	40.71 a	3.5 a	817.82 a	49.72 a
Total/Plant	98.9 A	8.29 A	2030.06 A	99.44 A
		40% profile & 50%T80:50%T70		
Harvest 1	27.01 a	2.19 a	566.81 a	18.94 b
Harvest 2	33.81 a	2.64 a	723.28 a	34.03 a
Harvest 3	26.86 a	2.85 a	494.47 a	37.57 a
Total/Plant	83 ABC[y]	7.69 A	1828.48 A	90.22 A
		100% profile & 50%T100:50%T70		
Harvest 1	33.07 a	2.34 b	673.59 a	24.88 a
Harvest 2	28.92 a	2.38 ab	613.56 a	31.03 a
Harvest 3	27.62 a	3.23 a	546.54 a	37.96 a
Total/Plant	92.4 AB	7.95 A	1919.18 A	98.8 A
		40% profile & 50%T80:50%T70		
Harvest 1	29.93 a	2.98 a	598.74 a	29.56 ab
Harvest 2	18.52 b	2.30 a	424.31 ab	25.86 b
Harvest 3	18.71 b	2.41 a	364.97 b	37.73 a
Total/Plant	67.16 C	7.70 A	1388.02 B	93.14 A
Substrate (*P*<0.05)	*	0.73	**	0.78
Fertlizer (*P*<0.05)	**	0.18	**	0.15
Substrate*Fertilizer (*P*<0.05)	0.35	0.4	0.25	0.4

[z]Mean separation among harvests within the same fertilizer treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y] Effect of fertilizer and substrate treatments on yield ($P< 0.05$), whereby means associated with different letters are significantly different

Table 9 Effect of harvest on mizuna mineral content. Plants grew in 100% Profile substrate and 50%T100:50%T70 controlled-release fertilizer. Each value is the average of three replicates.

Mineral		Harvest 1		Harvest 2		Harvest 3	
N[y]	**(%)**	7.5	a[z]	6.0	b	4.3	c
S	**(%)**	1.3	ab	1.6	a	1.0	b
P	**(%)**	0.5	a	0.5	a	0.7	a
K	**(%)**	5.5	a	4.9	a	3.5	b
Mg	**(%)**	0.6	a	0.7	a	0.7	a
Ca	**(%)**	1.5	a	1.4	a	1.1	b
Na	**(%)**	0.2	b	0.3	a	0.2	b
B	**(ppm)**	133.3	b	194.0	a	162.0	ab
Zn	**(ppm)**	38.0	a	49.3	a	64.3	a
Mn	**(ppm)**	2198.7	a	2313.7	a	2378.0	a
Fe	**(ppm)**	108.0	a	254.0	a	420.7	a
Cu	**(ppm)**	6.0	b	8.7	b	11.0	a
Al	**(ppm)**	5.7	b	25.0	b	75.3	a

[z]Mean separation among treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum

Table 10 Effect of harvest on mizuna mineral content. Plants grew in 100% Profile substrate and 50%T180:50%T70 controlled-release fertilizer. Each value is the average of three replicates.

Mineral		**Harvest 1**		**Harvest 2**		**Harvest 3**	
N[x]	**(%)**	7.7	a[z]	4.2	b	3.5	c
S	**(%)**	1.1	a	1.8	b	1.0	a
P	**(%)**	0.5	b	0.6	ab	0.6	a
K	**(%)**	5.5	a	4.2	b	2.7	c
Mg	**(%)**	0.6	b	0.8	ab	1.0	a
Ca	**(%)**	1.4	a	1.4	a	1.5	a
Na	**(%)**	0.2	a	0.2	a	0.1	b
B	**(ppm)**	121.7	a	207.7	b	212.7	b
Zn	**(ppm)**	41.3	b	47.7	ab	58.7	a
Mn	**(ppm)**	1953.7	b	2595.0	b	3763.7	a
Fe	**(ppm)**	124.0	a	102.0	a	148.3	a
Cu	**(ppm)**	12.0	a	8.7	a	10.7	a
Al	**(ppm)**	7.7	b	41.3	b	162.3	a

[z]Mean separation among treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum

Table 11 Effect of harvest on mizuna mineral content. Plants grew in 40% profile substrate and 50%T100:50%T70 slow release fertilizer. Each value is the average of three replicates.

Mineral		Harvest 1		Harvest 2		Harvest 3	
N[y]	**(%)**	8.1	a[z]	5.8	b	3.6	c
S	**(%)**	1.2	a	0.8	b	0.6	b
P	**(%)**	0.8	a	0.7	b	0.5	c
K	**(%)**	5.3	a	3.6	b	2.3	c
Mg	**(%)**	0.6	b	0.7	b	0.9	a
Ca	**(%)**	0.9	a	0.9	a	0.9	a
Na	**(%)**	0.14	a	0.14	a	0.07	b
B	**(ppm)**	187.3	a	204.7	a	146.7	b
Zn	**(ppm)**	48.0	b	65.0	ab	68.7	a
Mn	**(ppm)**	1839.9	c	2249.0	b	2977.67	a
Fe	**(ppm)**	142.3	b	164.7	b	215.33	a
Cu	**(ppm)**	9.9	a	12.0	a	11.67	a
Al	**(ppm)**	11.9	a	74.3	a	185.00	a

[z]Mean separation among treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum

Table 12 Effect of harvest on mizuna mineral content. Plants grew in 40% profile substrate and 50%T180:50%T70 slow release fertilizer. Each value is the average of three replicates.

Mineral		**Harvest 1**		**Harvest 2**		**Harvest 3**	
N[y]	**(%)**	5.8	a[z]	3.7	b	3.0	b
S	**(%)**	1.0	a	0.7	b	0.6	b
P	**(%)**	0.6	a	0.4	a	0.4	a
K	**(%)**	4.4	a	2.5	b	1.9	b
Mg	**(%)**	0.6	b	0.9	ab	1.1	a
Ca	**(%)**	0.9	a	1.1	a	1.1	a
Na	**(%)**	0.1	a	0.1	a	0.1	a
B	**(ppm)**	171.0	a	176.7	a	148.3	a
Zn	**(ppm)**	46.3	a	56.7	a	67.0	a
Mn	**(ppm)**	1985.7	b	2711.3	ab	3593.3	a
Fe	**(ppm)**	124.0	b	148.0	a	347.0	a
Cu	**(ppm)**	8.7	b	10.7	ab	12.0	a
Al	**(ppm)**	39.0	b	77.0	b	251.3	a

[z]Mean separation among treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

[y](N) nitrogen, (S) sulfur, (P) phosphorus, (K) potassium, (Mg) Magnesium, (Ca) Calcium, (Na) Sodium, (B) boron, (Zn) zinc, (Mn) Manganese, (Fe) iron, (Cu) Copper, (Al) Aluminum

Table 13 Effect of fertilizer and substrate on mizuna growth parameters (fresh weight, dry weight, leaf area and leaf number). Each value is the average of three replicates.

Substrate	Fertilizer	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Leaf Number
First harvest					
100% profile	**50%T100:50%T70**	23.64 a[z]	2.05 a	501.84 a	18.1 b
	50%T180:50%T70	27.01 a	2.2 a	566.81 a	18.94 b
40%profile	**50%T100:50%T70**	33.07 a	2.34 a	673.59 a	24.87 ab
	50%T180:50%T70	29.92 a	2.98 a	598.74 a	29.56 a
Second harvest					
100% profile	**50%T100:50%T70**	35.94 a	2.86 a	741.06 a	32.72 ab
	50%T180:50%T70	33.81 ab	2.64 a	723.28 ab	34.03 a
40%profile	**50%T100:50%T70**	28.92 b	2.38 a	613.56 b	31.03 ab
	50%T180:50%T70	18.52 c	2.31 a	424.31 c	25.86 b
Third harvest					
100% profile	**50%T100:50%T70**	40.71 a	3.5 a	817.82 a	49.72 a
	50%T180:50%T70	26.86 b	2.85 a	494.47 b	37.57 a
40%profile	**50%T100:50%T70**	27.62 ab	3.23 a	546.53 ab	37.96 a
	50%T180:50%T70	18.71 b	2.41 a	364.97 b	37.73 a

[z]Mean separation among treatments by Tukey HSD ($P< 0.05$), whereby means associated with different letters are significantly different

Table 14 Effect of fertilizer and substrate on mizunamineral content. Each value is the average of three replicates.

Substrate	Fertilizer	N (%)	S (%)	P (%)	K (%)	Mg (%)	Ca (%)	Na (%)	B (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Al (ppm)
First harvest														
100% profile	**50%T100:50%T70**	7.5 a[z]	1.3 a	0.53 b	5.5 a	0.62 a	1.5 a	0.20 a	133.3 ab	38.0 a	2198.6 a	108 b	6.0 a	5.7 b
	50%T180:50%T70	7.6a	1.3 a	0.57 b	5.3 a	0.61 a	1.4 a	0.19 a	133.4 b	41.2 a	2165.8 a	122.8 ab	10.4 a	9.2 b
40%profile	**50%T100:50%T70**	8.1 a	1.2 a	0.81a	5.3 a	0.62 a	0.9 a	0.15 ab	211.3 a	52 a	1756.5 a	150.3 a	11.5 a	14.5 b
	50%T180:50%T70	5.8 b	1.0 a	0.59 b	4.4 a	0.63 a	0.9 a	0.11 b	171.0 ab	46.3 a	1985.6 a	124 ab	8.7 a	39.0 a
Second harvest														
100% profile	**50%T100:50%T70**	6.0 a	1.6 a	0.55ab	4.9 a	0.69 a	1.4 a	0.29 a	194.0 a	49.3 ab	2313.7 a	254.0 a	8.7 b	25.0 b
	50%T180:50%T70	4.9 ab	2.0 a	0.64 a	4.4 a	0.75 a	1.5 a	0.20 b	213.0 a	47.4 b	2800.8 a	113.4 a	9.4 b	34.6 b
40%profile	**50%T100:50%T70**	5.82 a	0.8 b	0.67 a	3.6 b	0.74 a	0.88 b	0.14bc	204.6 a	65 a	2249 a	164.6 a	12 a	74.3
	50%T180:50%T70	3.7 b	0.7 b	0.44 b	2.6 b	0.92 a	1.1 b	0.08 c	176.7 a	56.7 ab	2711.3 a	148 a	10.7ab	77.0 a
Third harvest														
100% profile	**50%T100:50%T70**	4.3 a	1.0 a	0.66 a	3.5 a	0.73 a	1.1 a	0.19 a	162.0 ab	64.3 a	2378.0 a	420.7 a	11.0 a	75.3 b
	50%T180:50%T70	3.6 ab	1.1 a	0.57 ab	2.7 b	0.88 a	1.3 a	0.10 b	196.6 a	53.0 a	3400.2 a	162.8 a	9.8 a	141.2 bc
40%profile	**50%T100:50%T70**	3.6 ab	0.56 b	0.5 ab	2.3 b	0.91 a	0.93 a	0.07 bc	146.7 b	68.7 a	2977.7 a	215.3 a	11.7 a	185 ac
	50%T180:50%T70	3.1 b	0.6 b	0.44 b	1.9 b	1.05 a	1.1 a	0.05 c	148.3 ab	67.0 a	3593.3 a	347.0 a	12.0 a	251.3 a
Substrate (*P*< 0.05)		NS[y]	***[x]	NS	*	NS	***	***	NS	**	NS	NS	NS	*
Fertilizer (*P*< 0.05)		*	NS	*	NS	NS	NS	***	NS	NS	*	NS	NS	NS
Substrate*Fertilizer (*P*< 0.05)		NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Mean separation among treatments by Tukey HSD (*P*< 0.05), whereby means associated with different letters are significantly different

[y]NS, non-significant[*, **, **] at *P≤0.05, 0.01,* or 0.001 respectively

Table 15 Effect of substrate &fertilizer on leachate pH and EC. Each value is the average of three replicates.

substrate	fertilizer	PH	EC
100% Profile	**50%T100: 50 %T70**	6.14	0.27
100% Profile	**50%T180: 50%T70**	5.91	0.37
40% Profile: 60 % Turface	**50%T100: 50%T70**	5.80	0.82
40% Profile: 60 % Turface	**50%T180: 50%T70**	5.53	0.43
Significance (*P*<0.05)		**NS**	**NS**

[y]NS, non-significant[*, **, **] at $P \leq 0.05$, *0.01*, or 0.001 reAaaspectively

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