Improving the Efficiency of Organic Fertilizer For Soilless Cultivation Using Plant Growth Promoting Microorganisms

by

Nicklas McClintic

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved November 2022 by the
Graduate Supervisory Committee:

Yujin Park, Chair
Christopher Ryan Penton
Changbin Chen

ARIZONA STATE UNIVERSITY

December 2022

ABSTRACT

Chemical fertilizers are commonly used for controlled environment agriculture because they provide essential plant nutrient efficiently. However, rising fertilizer costs, global phosphorous shortage, and the negative impacts of producing and using chemical fertilizer are concerns for sustainable crop production. As sustainable alternatives to chemical fertilizers, there is a growing interest in using organic fertilizers with beneficial plant growth promoting microorganisms. The objectives of this research were to investigate how the application of plant growth promoting bacteria and arbuscular mycorrhizal fungi influences plant growth of lettuce (*Lactuca sativa*) and tomato (*Solanum lycopersicum*) seedlings in soilless media under organic fertilization. In the first study, the effects of *Azosprillium brasilense* and *Rhizophagus intraradices* inoculation on lettuce and tomato seedling growth were quantified under two different organic fertilizer types compared to under chemical fertilizer. The results showed that *A. brasilense* and *R. intraradices* had little to no effect on any growth parameter measured in either species regardless of fertilizer type. In the second study, an investigation of the co-inoculation of *A. brasilense* and *R. intraradices* or increasing the application frequency of *A. brasilense* or/and *R. intraradices* increased plant growth promoting effects in lettuce ‘Cherokee’ and ‘Rex’ grown under organic fertilization. An application frequency of every 2-days of the *R. intraradices* without or with *A. brasilense* increased shoot fresh weight in both lettuce cultivars by 51-58%, compared to un-inoculated control. In contrast, lettuce seedling growth were similar without or with applying *R. intraradices* weekly or *A. brasilense* regardless of frequency. Together, the results suggest that applying *R. intraradices* with a proper application frequency can enhance plant growth of lettuce under organic fertilization.

ACKNOWLEDGMENTS

I would like to greatly thank Dr. Yujin Park as my advisor for the enormous amount of guidance, support, and horticultural expertise that she has passed down to me throughout my graduate career. Most of if not all my achievements in my graduate career were thanks to Dr. Yujin Park's encouragement for me to move outside of my comfort zone to improve myself.

I would also like to thank my graduate committee members Dr. Christopher Ryan Penton, for providing microbiological analysis, and Dr. Changbin Chen, for his suggestions to improve my experimental work. A special thanks to Dr. Lin Li for her support throughout my thesis development process, and to Zhihao Chen for his contribution to the vertical farm and food waste fertilizer.

Furthermore, I would like to thank the following graduate and undergraduate students for their support and friendship: Johnathan Ries, Chris Beben, Ethan Larson, Tristan Lewis, Jasmine Goode, Lacy Hall. Lastly, I would like to thank my family for their support, encouragement, and guidance throughout the ups and down's that comes with pursing a master's degree. Finally, I wish to thank everybody at the CISA college and AZCati department for their assistances and encouragement for pursuing a higher education.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

Table Page

Table Page

Table Page

LIST OF FIGURES

Figure Page

LIST OF SYMBOLS / NOMENCLATURE

Symbol Page

1. AMF, Arbuscular Mycorrhizal Fungi; DW, Shoot Dry Weight; FW, Shoot Fresh Weight; LEDs, Light-emitting Diodes; N, Nitrogen; PGPB, Plant Growth Promoting Bacteria; PGPMs, Plant Growth Promoting Microorganisms; PPFD, Photosynthetic Photon Flux Density; SPAD, Soil-plant Analysis Development……………………………..17
2. AD, Anerobic Digestate; AMF, Arbuscular Mycorrhizal Fungi; ANOVA, Analysis of Variance; DLI, Daily Light Integral; Dw, Shoot Dry Weight; EC, Electrical Conductivity; FW, Shoot Fresh Weight; K, Potassium; LEDs, Light Emitting Diodes; N, Nitrogen; P, Phosphorus; PGPB, Plant Growth Promoting Bacteria; PGPMs, Plant Growth Promoting Microorganisms; PPFD, Photosynthetic Photon Flux Density; SPAD, Soil-plant Analysis Development………..…..52

CHAPTER 1

LITERATURE REVIEW

LITERATURE REVIEW

Hydroponics

Hydroponics refer to growing plants in a nutrient solution with or without the use of a soilless medium (Sharma et al., 2018). Different types of media can be used to provide a foundation for plant growth including gravel, vermiculite, rockwool, peat moss, saw dust, coir dust, and coconut fiber. Hydroponics enable precise controls of root-zone environments, such as nutrient concentration, pH, dissolved oxygen, and temperature, which all affect plant growth and nutrient uptake (Kozai et al., 2019). Compared to traditional soil-based farming, the benefits of using hydroponics often include higher yields, reduced crop production time, higher crop quality, water conservation, higher benefit to cost ratios, and reduced need for arable soil (Majid et al., 2020).

Among several hydroponic systems, deep-water culture (DWC) and nutrient film technique (NFT) are most used for commercial production (Sharma et al., 2018). In a DWC system, plant roots are pendulated in a nutrient solution to receive an uninterrupted quantity of water, oxygen, and nutrients (Aires, 2018). To prevent the plant roots from downing in a DWC system, air pumps are employed to oxygenate the nutrient solution (Aires, 2018). NFT systems pump nutrient solutions through channels and return the solution to the starting point of the system. NFT recirculates the initial nutrient solution analogous to a DWC system, however in an NFT system, the plant roots are not completely immersed in the nutrient solution (Aires, 2018). DWC systems are more favored because it is simpler and more inexpensive to install and use DWC over NFT (Majid et al., 2020).

Nutrient solutions

Nutrient solutions in hydroponic systems need to supply 14 essential elements, including six macronutrients [nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg)] and eight micronutrients at adequate concentrations throughout the life cycle of the crop (Hochmuth and Hochmuth, 2001, Wada, 2019). Synthetic inorganic fertilizers have been commonly used to formulate hydroponic nutrient solutions with multiple advantages because the composition and concentrations of macro and micronutrients can be easily refined with inorganic fertilizer; the inorganic forms of macro and micronutrients are immediately available for plant uptake; and chemical fertilizers can maintain a stable pH (Saeed et al., 2015). However, chemical fertilizers can cause environmental problems such as fertilizer run-off into oceans causing algal blooms as well as contaminating groundwater (Paull, 2009; Zaman et al, 2009).

Some recent studies report the potential of using sustainable organic fertilizers for hydroponic crop production. Phibunwatthanawong and Riddech (2019) have demonstrated that Green Cos lettuce (*L. sativa* var. *longifolia*) can be grown hydroponically using liquid organic fertilizers produced from distillery slop, molasses, and sugarcane leaves. Compared to Green Cos lettuce grown with chemical fertilizer, those grown with organic fertilizers for 28 days had greater root length at least partly associated with the existence of beneficial microorganisms and indole-acetic-acid (IAA) in organic fertilizers (Phibunwatthanawong and Riddech, 2019). In another study, fish-based organic fertilizers in combination with Japanese maple tree bark compost as a microbial inoculate increased lettuce (*Lactuca sativa* var. *capitata*) shoot fresh weight compared to that grown with conventional fertilizer although this trend was not observed in tomato (*Solanum lycopersicum* cv. 'Ponderosa') (Shinohara et al., 2011). In bok choy (*Brassica rapa* var. Chinensis), the plants grown with corn steep liquor or conventional chemical fertilizer at the same amount of total N had similar dry weight of leaves and nutrient concentration, including K, Ca, and Mg (Kano et al., 2021).

Plant growth promoting microorganisms

Plant growth-promoting microorganisms (PGPMs) include symbiotic bacteria and fungi that can provide benefits such as modulating the production of phytohormones, increase the availability of soil nutrients, and protecting their host plants from disease and abiotic stresses using a variety of plant-interaction mechanisms (Abhilash et al., 2016; Asghari et al., 2020; Souza et al., 2015; Etesami, 2020). Some genera of plant growth promoting bacteria (PGPB) used in commercial agriculture productivity include *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, *Frankia*, *Klebsiella*, *Clostridium*, *Trichoderma*, *Beauveria*, *Serratia*, and *Streptomyces* (Abhilash et al., 2016; Van Oosten et al., 2017; Gouda et al., 2018). Genera for arbuscular mycorrhizal fungi (AMF) include *Acaulospora, Entrophospora, Gigaspora, Scutellospora, Glomus, Rhizophagus, and Sclerocystis* (Dodd et al., 2000). Two of the most studied plant growth promoting microorganisms include PGPB

Azospirillum brasilense and AMF *Rhizophagus intraradices* formerly known as *Glomus intraadices.*

Azospirllum brasilense

Azospirllium are plump, slightly curved rod gram-negative bacteria with a single polar flagellum around 0.6-1.7 × 2.1-3.8 µm in size (Baldani et al., 2015). The optimal temperature for bacterial growth varies from 33 to 41°C and pH from 5.5 to 7.5 (Baldani et al., 2015). *Azospirillium* bacterium can fixate atmospheric N into usable forms for plant roots (Steenhoudt and Vanderleyden, 2000). In biological N fixation, nitrogenase is the catalyst enzyme for the biochemical system (Kim and Rees, 1994). The mechanisms which *Azospirillium* use to regulate nitrogenase activity is heavily dependent on oxygen concentration, N availability, and type of strain. *Azospirillium* spp. enzyme activity is most available at lower oxygen levels and become less active as oxygen levels increase (Vande Broek et al., 1996). For *A. Brasiliense,* the optimum nitrogenase activity occurred between 1%-2% of oxygen (Vande Broek et al., 1996). In contrast, when *A. brasilense* is grown in an anaerobic environment below 1-2% of oxygen, N fixation structural genes can dissimilate nitrate to nitrite or to nitrous oxide and N gas (Baldani et al., 2015; Steenhoudt and Vanderleyden, 2000).

The beneficial effects of *Azospirllum brasillense* inoculation on plant growth and development have been reported in a diverse array of crops. For example, wheat (*Triticum aestivum* L.) inoculated with specific *A. brasilense* stain produced an increase in shoot biomass and total plant N content compared to a disinfected control, showing that an increase of N fixed by *A. brasilense* positively correlated with an increase of NO_3^- in plant tissue (Saubidet et al., 2002). When lettuce seeds were inoculated with *Azospirillum* sp., germination percentages and seedling biomass increased under saline conditions compared to non-inoculated control (Barassi et al., 2006). In addition, inoculation of *A. brasilense* to pearl millet (*Pennisetum Americanum* L.) roots increased the number of lateral roots and root hair growth thereby increasing root fresh weight by nearly 20% (Tien et al., 1979). It has been known that some *Azosprillium* species can promote root growth by the secretion of phytohormones such as indole-3-acetic acid. Indole-3-acetic acid. Indole-3-acetic acid (IAA) is a plant hormone that alters the carbohydrates in plant cell walls allowing for the formation of new cell wall material (Britannica, 2021). A symbiotic mechanism known as bacterial IAA synthesis where the plant secretes tryptophan to stimulate the growth of root-colonizing bacterium to enhance IAA synthesis by the rhizobacterium (Keswani et al., 2020). A common *Azospirillium* inoculation method to increase the colonization success and obtain the beneficial effects has been to soak the seeds in the bacterial suspension before planting (Mangmang et al., 2015). Inoculating seeds with *Azospirillum brasillens* by soaking was generally a little more effective in promoting germination and seedling growth in tomato (*Lycopersicon esculentum* L., cv. Grosse lisse) than drenching (Mangmang et al., 2015).

Rhizophagus intraradices

The primary mechanism of AMF development on the root surface (ectomycorrhizas) or within the roots (ericoid and arbuscular mycorrhizas) is to grow and extend hyphae that penetrate root cell pores thus increasing the uptake of water and nutrients like N from organic sources (Kuzyakov and Xu, 2013). It is thought that mycorrhizal hyphae contribute nearly 30% of N accumulation to its host plant (Xie et al., 2021). AMF that colonizes inside the root cortex of host plants and form highly branched structures inside the cells are known as arbuscules, which are considered the exchange site of mineral nutrients for the plants in return for carbohydrates, and vesicles which are specialized fungi sites for storing carbohydrates (Berruti et al., 2016; Balestrini et al., 2015).

Rhizophagus intraradices can aid in the fixation of N. Its ability to N fixation is affected by environmental factors, such as available P and carbon (C) (Soretire et al., 2020; Xie et al., 2021). AMF uses P as a catalyst to absorb both NO_3 and NH_4, in the production of plant metabolites in exchange for C that AMF uses to proliferate hyphae and produce more metabolites (Bücking and Kafle, 2015). Optimum P levels promote AMF nodules in return increasing plant shoot and root dry weight. For example, Mohammad et al. (2004) investigated the growth potential of wheat (*Triticum aestivum* var. Swift) inoculated with AMF that were fertilized with different amounts of P. Their results show plants that were given P fertilizer between 5-10 kg ha^{-1} had significantly increased mycorrhizal growth leading to improved crop productivity in both shoot dry weight, and number of grains per spike compared to control plants. The study suggests that plant growth can be attributed to early AMF colonization of the roots leading to increased efficiency of P absorption enhancing plant growth (Mohammad et al., 2004). In contrast, excessive or inadequate amounts of P can prevent the activation of N fixing enzymes over time (Mohammad et al., 2004; Soretire et al., 2020). Soretire et al. (2020) reported that soybean plants (*Glycine max* L.) fertilized with P rates of 20 kg ha^{-1} and infected with *R. intraradices* increased shoot and root biomass compared to plants fertilized with P at 40 kg ha^{-1}. It has been documented that colonization rate of *R. intraradices* positively correlated with a total C and N content, suggesting that host plants that receive more N will exchange higher rates of C to its symbiotic fungi growing on the plants roots (Xie et al., 2021).

AMF have evolved in conjunction with plants to produce plant hormone compounds, such as IAA and various gibberellic acids, to easily penetrate plant cells and favor growth without activating the plant's basal immunity to protect themselves from a possible fungal infection. Gibberellic acid (GA) is a naturally occurring plant hormone that is also present in fungi, and its role as a plant growth regulator is to stimulate cell division and elongation in roots and shoots. Trifoliate orange (*Citrus trifoliata*) seedlings infected with *R. intraracides* had higher amounts of root IAA and greater root hair density and average root hair diameter compared to non-infected plants (Wu et al., 2016). Similarly, fungal-produced IAA stimulated a better root system formation, enhancing water and nutrient uptake in crop plants like maize (*Zea mays*) and rice (*Oryza sativa*) (Keswani et al., 2020). It has been documented that some plants, such as tobacco (*Nicotiana tabacum*) and birdsfoot (*Lotus japonicsu*) infected with AMF show increases in GA in the roots resulting in increased root diameter (Shaul-Keinan et al., 2002).

REFERENCES

Abhilash, P. C., Dubey, R. K., Tripathi, V., Gupta, V. K., & Singh, H. B. (2016). Plant growth-promoting microorganisms for environmental sustainability. *Trends in Biotechnology*, *34*(11), 847-850.

Aires, A. (2018). Hydroponic production systems: Impact on nutritional status and bioactive compounds of fresh vegetables. *Vegetables: Importance of Quality Vegetables to Human Health*, *55*.

Asghari, B., Khademian, R., & Sedaghati, B. (2020). Plant growth promoting rhizobacteria (PGPR) confer drought resistance and stimulate biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage condition. *Scientia Horticulturae*, *263*, 109132.

Baldani, J. I., Krieg, N. R., Baldani, V. L. D., Hartmann, A., & Döbereiner, J. (2015). Azospirillum. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1-35.

Balestrini, R., Lumini, E., Borriello, R., & Bianciotto, V. (2015). Plant-soil biota interactions. *Soil Microbiol Ecol Biochem*, 311-338.

Barassi, C. A., Ayrault, G., Creus, C. M., Sueldo, R. J., & Sobrero, M. T. (2006). Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. *Scientia Horticulturae*, *109*(1), 8-14.

Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in microbiology*, *6*, 1559.

Britannica, T. 2021. Information Architects of Encyclopedia. Encyclopedia Brit. https://www.britannica.com/facts/auxin

Bücking, H., & Kafle, A. (2015). Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. *Agronomy*, *5*(4), 587-612.

Dodd, J. C., Boddington, C. L., Rodriguez, A., Gonzalez-Chavez, C., & Mansur, I. (2000). Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function, and detection. *Plant and soil*, *226*(2), 131-151.

Etesami, H. (2020). Plant–microbe interactions in plants and stress tolerance. In *Plant Life Under Changing Environment* (pp. 355-396). Academic Press.

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological research*, *206*, 131-140.

Hochmuth, G. J., & Hochmuth, R. C. (2001). Nutrient solution formulation for hydroponic (perlite, rockwool, NFT) tomatoes in Florida. *HS796. Univ. Fla. Coop. Ext. Serv., Gainesville*, 1-10.

Kano, K., Kitazawa, H., Suzuki, K., Widiastuti, A., Odani, H., Zhou, S., ... & Sato, T. (2021). Effects of organic fertilizer on bok choy growth and quality in hydroponic cultures. *Agronomy*, *11*(3), 491.

Keswani, C., Singh, S. P., Cueto, L., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., ... & Sansinenea, E. (2020). Auxins of microbial origin and their use in agriculture. *Applied Microbiology and Biotechnology*, *104*(20), 8549-8565.

Kim, J., & Rees, D. C. (1994). Nitrogenase and biological nitrogen fixation. *Biochemistry*, *33*(2), 389-397.

Kozai, T., Niu, G., & Takagaki, M. (Eds.). (2019). *Plant factory: an indoor vertical farming system for efficient quality food production*. Academic press.

Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytologist*, *198*(3), 656-669.

Majid, M., Khan, J. N., Shah, Q. M. A., Masoodi, K. Z., Afroza, B., & Parvaze, S. (2021). Evaluation of hydroponic systems for the cultivation of Lettuce (*Lactuca sativa* L., var. Longifolia) and comparison with protected soil-based cultivation. *Agricultural Water Management*, *245*, 106572.

Mangmang, J. S., Deaker, R., & Rogers, G. (2015). Early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated by soaking and drenching. *Horticultural Science*, *42*(1), 37-46.

Mohammad, A., Mitra, B., & Khan, A. G. (2004). Effects of sheared-root inoculum of *Glomus intraradices* on wheat grown at different phosphorus levels in the field. *Agriculture, ecosystems & environment*, *103*(1), 245-249.

Phibunwatthanawong, T., & Riddech, N. (2019). Liquid organic fertilizer production for growing vegetables under hydroponic condition. *International Journal of Recycling of Organic Waste in Agriculture*, *8*(4), 369-380.

Paull, J. (2009). A century of synthetic fertilizer: 1909-2009.

Saeed, K. S., Ahmed, S. A., Hassan, I. A., & Ahmed, P. H. (2015). Effect of bio-fertilizer and chemical fertilizer on growth and yield in cucumber (*Cucumis sativus*) in green house condition. *Pak J Biol Sci*, *18*(3), 129-134.

Saubidet, M. I., Fatta, N., & Barneix, A. J. (2002). The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant and soil*, *245*(2), 215-222.

Sharma, N., Acharya, S., Kumar, K., Singh, N., & Chaurasia, O. P. (2018). Hydroponics as an advanced technique for vegetable production: An overview. *Journal of Soil and Water Conservation*, *17*(4), 364-371.

Shaul-Keinan, O., Gadkar, V., Ginzberg, I., Grünzweig, J. M., Chet, I., Elad, Y., ... & Kapulnik, Y. (2002). Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with Glomus intraradices. *New Phytologist*, *154*(2), 501-507.

Shinohara, M., Aoyama, C., Fujiwara, K., Watanabe, A., Ohmori, H., Uehara, Y., & Takano, M. (2011). Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. *Soil science and plant nutrition*, *57*(2), 190-203.

Soretire, A. A., Adeyemi, N. O., Atayese, M. O., Sakariyawo, O. S., & Adewunmi, A. (2020). Nodulation and biological nitrogen fixation in soybean (*Glycine max* L.) as influenced by phosphorus fertilization and arbuscular mycorrhizal inoculation. *Acta Universitatis Sapientiae, Agriculture and Environment*, *12*(1), 22-44.

Souza, R. D., Ambrosini, A., & Passaglia, L. M. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and molecular biology*, *38*, 401-419.

Steenhoudt, O., & Vanderleyden, J. (2000). *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS microbiology reviews*, *24*(4), 487-506.

Tien, T. M., Gaskins, M. H., & Hubbell, D. (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and environmental microbiology*, *37*(5), 1016-1024.

Vande Broek, A., Keijers, V., & Vanderleyden, J. (1996). Effect of oxygen on the free-living nitrogen fixation activity and expression of the *Azospirillum brasilense* NifH gene in various plant-associated diazotrophs. *Symbiosis*.

Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., & Maggio, A. (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*, *4*(1), 1-12.

Wada, T. (2019). Theory and technology to control the nutrient solution of hydroponics. In *Plant Factory Using Artificial Light* (pp. 5-14). Elsevier.

Wu, Q. S., Liu, C. Y., Zhang, D. J., Zou, Y. N., He, X. H., & Wu, Q. H. (2016). Mycorrhiza alters the profile of root hairs in trifoliate orange. *Mycorrhiza*, *26*(3), 237-247.

Xie, M. M., Chen, S. M., Zou, Y. N., Srivastava, A. K., Rahman, M. M., Wu, Q. S., & Kuča, K. (2021). Effects of *Rhizophagus intraradices* and *Rhizobium trifolii o*n growth and N assimilation of white clover. *Plant Growth Regulation*, *93*(3), 311-318.

Zaman, S., Pramanick, P., & Mitra, A. (2019). Chemical fertilizer. *Department of Marine Science, University of Calcutta: Kolkata, India*.

CHAPTER 2

QUANTIFYING GROWTH RESPONSES OF LETTUCE AND TOMATO SEEDLINGS TO PLANT GROWTH-PROMOTING BACTERIA AND MYCORRHIZAL FUNGI INOCULATION UNDER DIFFERENT FERTILIZER TYPES

ABSTRACT

There is an increasing interest of using organic fertilizers and plant growth-promoting microorganisms (PGPMs) as sustainable alternative to chemical fertilizer for horticultural crop production. However, a variety of factors, including fertilizer types, PGPM species, and plant species, can influence the effectiveness of PGPM inoculation and organic fertilizer. In the present study, the effects of *Azospirillium brasilense* and *Rhizophagus intraradices* inoculation in combination with different fertilizer types were evaluated on the seedling growth of soilless lettuce (*Lactuca sativa* 'Cherokee') and tomato (*Solanum lycopersicum* 'Red Robin'). In a temperature-controlled vertical farm, the seeds of lettuce and tomato were sown in rockwool substrate, and *A. brasilense* (1.05 × 10^8 CFU/L) or *R. intraradices* (580 propagules/L) were applied through sub-irrigation weekly at and after sowing. The seedlings were fertilized with chemical fertilizer, fish-based organic fertilizer, and organic fertilizer derived from food waste at 100 ppm total nitrogen and grown at 23°C under sole-source LED lighting at a photosynthetic photon flux density of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 18-hour photoperiod. At the transplant stage, lettuce seedlings had 75% less shoot fresh weight and 64% less dry weight under organic fertilizers than chemical fertilizer. Similarly, tomato seedlings grown with organic fertilizers had one less leaf, 36% smaller stem diameter, 40% shorter stem length, and 75% or 67% less shoot fresh or dry weight, respectively, compared to seedlings grown with chemical fertilizer. In contrast, there was little to no effects of *A. brasilense* or *R. intraradices* inoculation seedling growth in both species. Further research is needed to determine the effective application methods of *A. brasilense,* and *R. intraradices* to have beneficial effects of using sustainable alternative fertilizers for indoor vegetable transplant production.

Keywords: anaerobically digested food waste, *Azospirillium*, beneficial microbe, controlled environment agriculture, chemical fertilizer, organic fertilizer, *Rhizophagus*

Abbreviations: AMF, Arbuscular Mycorrhizal Fungi; DW, Shoot Dry Weight; FW, Shoot Fresh Weight; LEDs, Light-emitting Diodes; N, Nitrogen; PGPB, Plant Growth Promoting Bacteria; PGPMs, Plant Growth Promoting Microorganisms; PPFD, Photosynthetic Photon Flux Density; SPAD, Soil-plant Analysis Development.

Introduction

Issues with using synthetic chemical fertilizers for growing food crops include greenhouse gas emissions from fertilizer production, soil phosphorus shortage, and soil and water pollution are all considered severe threats to sustainable food production (Nosheen et al., 2021; Oelkers and Valsami-Jones, 2008; Savci, 2012). Nearly 30-50% of the food produced using chemical fertilizers is not consumed by humans, resulting in a significant waste of natural resources, energy, and fertilizer. Additionally, food waste in landfills produce greenhouse gases which further contribute to air pollution and groundwater contamination (Buzby et al., 2014; Jurgilevich et al., 2016). One proposed solution to combat the inefficiency and negative environmental impacts of today's traditional food system model is a transition towards a circular food system, which recycles food wastes, by-products, and nutrients (Jurgilevich et al., 2016). Therefore, the production and use of sustainable environmental-friendly fertilizers derived from food waste in a circular food system is starting to be recognized as a sustainable agriculture solution (Chojnacka et al., 2020). Recently, anaerobically digested food waste has been demonstrated to be an effective fertilizer for a variety of food crops, such as bok choy (Pelayo Lind et al., 2021), tomato (Mickan et al., 2022; Stoknes et al., 2017), lettuce and other leafy vegetables (Nosheen et al., 2021; Stoknes et al., 2019).

Using organic fertilizers has multiple advantages, such as recycling nutrients, supplying beneficial organic bio-stimulants, and decreasing the demand for minerals used to produce chemical fertilizers (Bergstrand et al., 2022). However, the effects of organic fertilizer can vary depending on the nutrient sources (Shinohara et al., 2011). In addition, nutrient management is more challenging with organic fertilizers than chemical fertilizers since many nutrients bound to organic substances are not immediately available for plant uptake and require microbially mediated mineralization processes (Bergstrand et al., 2022; Watts-Williams et al., 2014). Using sterile and inert soilless substrates naturally do not support a robust microbial community, making organic fertilizer even more challenging to supply sufficient nutrients during soilless crop cultivation (Grunert et al., 2016).

One suggested method of increasing the effectiveness of organic fertilizers is harnessing the abilities of PGPMs (Bartelme et al., 2018; Paradiso et al., 2017; Sheridan et al., 2017). PGPMs are free-living or symbiotic bacteria and fungi that colonize plant roots and provide benefits to their host plants by promoting mineralization and solubilization of nutrients, regulating phytohormone production, and improving resistance to environmental stresses (Batista and Singh, 2021; Lopes et al., 2021; Souza et al., 2015). Some popular species of PGPMs for commercial use include the PGPB species *Azospirillum brasilense* and AMF species *Rhizophagus intraradices*. *Azospirillium brasilense* is an associative/free-living bacterium that can fix N under low oxygen environments (Hernaández-Esquivel et al., 2021). Bacteria belonging to the genus *Azospirillium* have been known to promote plant growth by solubilization of nutrients, synthesize plant hormones such as auxins and cytokinins, and produces siderophores for increased uptake of nutrients (Lopes et al., 2021). *Rhizophagus intraradices,* as an AMF, colonize plant root cells by forming arbuscules for the exchange of nutrients with their host plants (Li et al., 2014). AMF aids in plant growth by enhancing the efficiency of plants to uptake water and nutrients, synthesizing plant hormones, and increasing the decomposition of organic materials (Begum et al., 2021). Previous studies also showed using PGPMs can also increase nutrient use efficiency and plant growth under chemical fertilizer conditions (Khanghahi et al., 2018).

However, while many abiotic factors affect the establishment and function of PGPM on plants, most studies on the effects of PGPM were conducted in soil and field conditions and relatively few studies were performed in soilless media under a controlled environment (Majeed et al., 2018; Mishra and Sundari, 2013; Mwashasha et al., 2016). In general, PGPM effects were greater under harsh or stressful environment as PGPMs employ to protect themselves and host plants from drought, salinity, and pathogens

(Balkrishna et al., 2022). Under soil conditions, PGPM effects were more prominent than in soilless conditions, because soil contains organic material that microbes can use for nutrient sources and rhizosphere environmental conditions are optimal for microbes. (Khare and Arora, 2014). Due to controlled environments having conditions that are most optimal for plant growth, PGPMs sometimes tend to outcompete plants for essential nutrients, such as nitrate and oxygen, negatively affecting overall plant growth (Macintyre et al., 2011; Moncada et al., 2021). Therefore, it is expected that the different environmental characteristics under the controlled environment and soilless environment will affect the interactions between plants and PGPM and subsequently, plant benefits derived by PGPM (de Haas, et al., 2021; Paradiso et al., 2017; Sheridan et al., 2017).

The objectives of this study were (1) to evaluate food waste anaerobic digestate as an organic fertilizer source for soilless cultivation of lettuce and tomato transplants compared to commercially available organic and chemical fertilizers and (2) to investigate how introducing *Azosprillum brasilense* and *Rhizophagus intraradices* in a soilless media influence lettuce and tomato seedling growth under organic and inorganic fertilizers.

Materials and Methods

Plant materials and growing environment

This study was performed two times, and in each replication, there were nine treatments (3 microorganism treatments × 3 fertilizer treatments). For each treatment, 40 rockwool cubes (A-OK Starter Plug 3.6 cm × 3.6 cm × 4.1 cm, Grodan, Milton, ON, Canada) were placed in a plastic plant tray (52.8 cm × 27.4 cm × 6.1 cm, Hawthorne, Vancouver, WA), and 20 seeds of tomato ‘Red Robin’ (Park Seeds, Greenwood, SC) and 20 seeds of lettuce ‘Cherokee’ (Johnny’s Selected Seeds, Winslow, ME) were sown into individual rockwool cubes. The seeded trays were covered with humidity domes and placed in a growing rack with three tiers (Fig 2.1). Three trays under the same fertilizer with three microorganism treatments were placed on the same tier of the growing rack. One week after sowing when both lettuce and tomato seeds germinated, the humidity domes were removed.

The seedlings were grown at a set air temperature of 23˚C under sole-source lighting from blue + red + white LEDs (T8 Double-Row LED Indoor Grow Light; Homer Farms Inc., Mesa, AZ) at a photosynthetic photon flux density (PPFD) of 200 µmol·m^{-2}·s^{-1} with an18-h photoperiod. PPFD was measured using a spectroradiometer ((PS-300,, StellerNet, Inc., Tampa, FL) by taking the average of nine predetermined points inside each growing rack tier with the quantum sensor measuring at seedling tray height. The temperature and relative humidity were monitored on the center of each tier of the growing rack at the seedling height using a hygrometer thermometer (H57075; Shenzhen Intellirocks Tech Co., Shenzhen, Guangdong, China) (Table 2.1).

Microorganism and fertilizer treatments

Microorganism treatments were applied at sowing and weekly after sowing. At sowing, rockwool cubes were presoaked, each three trays with tap water, AMF solution, or PGPB solution (Table 2.2). The AMF solution was made with *Rhizophagus* *intraradices* at a concentration of 580 propagules/L by hand mixing 2.36 g of mycorrhizal inoculum wettable powder (Mykos mycorrhizal wettable powder; Reforestation Technologies International; Gilroy, CA) per 1L of tap water. PGPB solution was made with *Azospirillium brasilense* at a concentration of 1.05 × 10^8 CFU/L by supplementing tap water with 1.05 mL liquid bacteria (Azos red liquid plant growth promoting bacteria; Reforestation Technologies International) per 1L of tap water. After sowing, the rockwool cubes were inoculated weekly through sub-irrigation with 1L of tap water, AMF solution, or PGPB solution.

One week after sowing when both lettuce and tomato seeds germinated, seedlings were irrigated as needed, every two or three days, through sub-irrigation with nutrient solutions made from tap water supplemented with chemical (15-5-20 Jack's Nutrients FeED, JR Peters, Allentown, PA), organic (3-3-2 AgroThrive Organic Bio-fertilizers; AgroThrive, Gonzales, CA), or food waste fertilizer (0.06-0.026-0.1191 Climate Saver, Homer Farms Inc.) at a 100 ppm N. Before each application, the pH of microorganism inoculation solutions and nutrient solutions were adjusted with a diluted (1:1) 95-89% sulfuric acid (J.Y. Baker, Inc) and potassium bicarbonate (Earthborn Elements; American Fork, UT), and the EC and pH of each solution were measured using a pH and EC meter (HI9814; Hannah Instruments; Woonsocket, RI) (Table 2.3).

Data collection and analysis

In each replication, the following seedling growth data were collected from 10 plants per treatment three weeks after sowing seeds: leaf number (leaf length ≥ 1.5 cm), leaf length and width for the first true leaf, relative chlorophyll concentration (SPAD index) [with a handheld SPAD meter (SPAD-502; Konica Minolta Sensing, Inc., Chiyoda, Tokyo, Japan)], shoot fresh and dry mass following ≥5 days in a drying oven (Hafo 1600 Oven, VWR International, LLC, Aurora, CO) at 86°C [with an analytical balance (PB602-S; Mettler Toledo, Columbus, OH)], plant diameter (lettuce only) with a ruler, stem diameter (tomato only) with a caliper device, and seedling height (tomato only) with a ruler.

The experiment used a randomized complete block design with two blocks and ten subsamples per block. Each replication was regarded as a block. Each plant tray was regarded as the experimental unit for microorganism and fertilizer treatments. Within each plant tray, ten individual seedlings per species were the sub-samples or observational units. Data were analyzed with the SAS (version 9.4; SAS Institute, Inc., Cary, NC) with the PROC MIXED procedure [with two fixed factors for microorganism and fertilizer treatments, two random factors of blocks and the interaction among blocks, microorganism treatments, and fertilizer treatments] that provided pairwise comparisons between treatments by using Tukey’s honestly significant test at $P<0.05$.

Results

Lettuce

There was little to no effects of microorganisms on lettuce seedling growth (Fig 2.2 and Table 2.5). Lettuce seedlings grown with chemical fertilizer had larger plant diameter (58-60%), leaf length (57-58%), leaf width (60-65%), shoot fresh and dry weight (117-128% and 92-104%, respectively) than those grown with organic or food waste fertilizer. Leaf number and SPAD index were generally similar among fertilizer treatments, although SPAD index was greater under food waste fertilizer with AMF than under chemical fertilizer. Organic and food waste fertilizer had similar effects on lettuce seedling growth.

Tomato

Microorganism treatments did not influence tomato seedling growth (Fig 2.3 and Table 2.6). Tomato seedlings grown with chemical fertilizer had 37-40% more leaves and greater shoot fresh and dry weight (115-134% and 94-115%, respectively) than those grown with organic or food waste fertilizer. Stem lengths were 47-52% longer and stem diameters were 43-54% wider under chemical fertilizer than under organic or food waste fertilizers. Leaf length with chemical fertilizer were larger than with organic or food waste fertilizer except for with PGPB, in which leaf lengths were similar with that with food waste fertilizer with AMF. Leaf widths with chemical fertilizer were greater than those with organic fertilizer or greater than or similar to with food waste fertilizer. In general, organic and food waste fertilizer had similar effects on leaf number, leaf size, leaf width, and shoot fresh and dry weight. SPAD index were similar regardless of microorganisms and fertilizer treatments.

Discussion

Effects of fertilizers

In this study, when lettuce and tomato seedlings were fertilized at the same 100 ppm N, shoot fresh (dry) weight of lettuce and tomato seedlings were 117-128% (92-104%) and 115-134% (94-115%) greater under chemical fertilizer than with organic or food waste fertilizer, respectively. Similarly, a few studies reported that at the same total nitrogen (N) level, plants have greater biomass with chemical fertilizer than with organic fertilizer. For example, in bok choy (*Brassica rapa* v. *chinensis* cv. 'Seitei Chingensai'), chemical fertilizer increased shoot fresh weight (dry weight) by 6-140% (3-10%) compared to corn steep liquor fertilizer at 260 and 520 ppm N, respectively (Kano et al., 2021). In a separate study, squash (*Cucurbita pepo* L. cv. 'F_1 Alata Yesili') under chemical fertilizer increased shoot and root fresh weights by 18% compared to liquid organic fertilizer containing organic chicken manure at 151 ppm N (Dasgan and Bozkoylu, 2007). Plant biomass accumulation positively correlates with N availability (Fichtner and Schulze, 1992). N is available to plants as either ammonium or nitrate, and most plants preferably uptake nitrate over ammonium (Li et al., 2013). Chemical fertilizers provide N in the form of nitrate or ammonium ions. However, N in organic fertilizer exists in the organic form that is needed to be converted into nitrate and ammonium for plant uptake (Bi et al., 2010; Gaskell and Smith, 2007; Smith and Hadley, 1989). In this study, 80% and 20% of the N in the chemical fertilizer were nitrate and ammonium, respectively. In contrast, most of the N in the food waste fertilizer existed in the ammonium (63%) and organic N (37%) forms, and the organic fertilizer derived from corn steep liquor and fermented fish by-products provided only 5% of the N in the ammoniacal N. Considering that plant growth increases with increasing N availability and organic N is not readily available for plant uptake, the stunted plant growth under organic fertilizers at the same N level could be at least partly attributed to the lower N availability or inorganic N concentration.

In this study plant growth effects of organic fertilizer and food waste fertilizer were similar. The results from this study agree with multiple studies involving cabbage (*Brassica oleracea* var. capitata) under different types of organic fertilizers having similar non-significant effects on plant growth parameters compared to chemical fertilizer (Kang et al., 2021; Zahradník and Petrikova, 2007). This study's results also agree with studies involving tobacco (*Nicotiana tabacum* L.) and mint (*Mentha* x *piperita* L.) fertilized with different types of organic fertilizers having similar effects on overall plant growth regardless of fertilizer source (Costa et al., 2013; Kurt and Ayan, 2014). Results from the studies could be due to nutrient deficiencies from non-readily available N forms in organic fertilizers that cannot be rapidly absorbed and require mineralization processes overtime unlike chemical fertilizers (Bergstrand, 2022; Chen, 2006; Phibunwatthanawong and Riddech, 2019).

Effects of PGPMs

The application of AMF and PGPB had little to no effect on plant growth in lettuce and tomato seedlings. The plant growth promoting effects of AMF and PGPB, mediated by increased nutrient uptake and plant hormone production, have been reported in a wide range of crops (Grobelak et a., 2015; Kim et al., 2017; Oljira et al., 2020; Shinohara et al., 2011). However, the beneficial effects of AMF and PGPB on plant growth can depend on rootzone temperature and pH because they can affect plant-microbe interactions (Agehara and Warncke, 2005; Gaskell and Smith, 2007; Moncada et al., 2020; Saijai et al., 2016; Silgram and Shepherd, 1999). In general, plant growth promoting effects of AMF and PGPB increased under warm rootzone temperature (25-40°C) and basic pH (7.5-8.5) as these conditions become more favorable for microbial activity (Curtin et al., 2012; Franzluebbers, 1999; Jones and Hood, 1980; Lopes et al., 2021; Linn and Doran, 1984; Saijai et al., 2016). In this study, we grew lettuce and tomato seedlings at the set air temperature of 23°C with an average nutrient solution pH of 5.8 principally for plant growth and nutrient uptake. Little effect of AMF and PGPB application could be due to lower temperature and lower pH than the ideal conditions, which can suppress the activities of AMF and PGPB.

The little effects of AMF and PGPB on plant growth could be a result of unsuccessful inoculation of lettuce and tomato seedlings with AMF and PGPM. The plant growth promoting effects of PGPMs require the successful inoculation of plants with microorganisms, which depends on inoculation method, microbe inoculant density, and root colonization (Lopez et al., 2021). For example, plant growth promoting effects from the PGPM *Phyllobacterium brassicaearum* showed dose-dependent responses with the maximum growth potential on roots of rapeseed (*Brassica napus*) from bacterial densities of around 10^8 CFU/mL. In addition, as microorganisms are usually immobile from the inoculation site to the rhizosphere, applying inoculums close to the root zone has been suggested (Lopez et al., 2021). In this study, inoculation densities were 580 propagules/L for AMF and 1.0 × 10^8 CFU/mL for PGPB. These propagation concentration densities could have been non-ideal with most successful plant growth effects from studies involving strains of *A. brasilense* with densities at 10^6 CFU/mL, and successful plant growth effects from *R. intraradices* with densities at 1000 propagules/g (Bashan and de-Bashan, 2002; Mangmang et al., 2015; Mikiciuk et al., 2019). AMF and PGPB were applied via the sub-irrigation. Relatively low inoculation density of AMF and PGPB and inoculation only via the sub-irrigation without direct inoculation into the seed and seedling roots possibly contributed to less successful establishment of PGPM population in the root zone and their growth promoting effects.

Biologically active root exudates range from discharged ions, free oxygen, water, enzymes, mucilage, and carbons compounds containing primary and secondary metabolites, such as amino acids and sugars (Hayat et al., 2017, Neumann et al., 2014). Root exudates act as key mechanisms for interactive communication signals between plant and microbes in the rhizosphere (Ortíz-Castro et al., 2009). For example, root exudates in arabidopsis (*Arabidopsis thaliana*), including malic acid, aided in the recruitment of PGPB *Bacillus subtilis* to seedling roots (Rudrappa et al., 2008). In tobacco (*Nicotiana tavacum*) and alfalfa (*Medicago sativa*), transgenic plants modified to overproduce root exudates showed increases in PGPM colonization and shoot biomass compared to control plants (Lopez-Bucio et al., 2000; Tesfaye et al., 2003). A major factor influencing root exudation production is plant age as seedings exudate smaller amounts compared to mature plants (Aulakh et al., 2001; Badri and Vivanco, 2009; Lucas

García et al., 2001). Given that the present study investigated the effects of AMF and PGPB inoculation during the seedling stage in lettuce and tomato, the small amounts of root exudates released from seedlings could be less effective to facilitate the plant-microbial interaction.

Conclusion

Here we evaluated organic fertilizer made from food waste and PGPMs as sustainable fertilizer options for indoor vegetable transplant production. At the same total nitrogen concentration, in both lettuce and tomato, seedling growth were similar under fish-based organic and food waste fertilizer, but under chemical fertilization, plants showed increased growth. With rockwool substrate, PGPM inoculants had little to no effect on lettuce and tomato seedlings growth, regardless of fertilizer type. Future research is needed to investigate how rootzone environment, PGPM inoculation method, and host-specificity influence the inoculation success and the beneficial effect of PGPMs on plant growth.

Figure 2.1. Image of Experimental Setup Inside Vertical Farm.

Table 2.1. Means (±SD) of Air Temperature (°C) and Relative Humidity (%) of Both Replications Inside of Growing Rack.

Location inside of growing rack	Air Temperature (°C)		Relative Humidity (%)	
	Replication 1	Replication 2	Replication 1	Replication 2
Top	22.2±1.72	24.2±1.96	63.7±10.7	60.9±10.4
Middle	22.7±1.96	23.8±1.83	58.9±10.4	64.6±10.1
Bottom	22.4±1.87	23.5±1.91	61.2±10.2	67.1±9.54

Table 2.2. Means (±SD) of Electrical Conductivity (EC) and pH of Control, Plant Growth Promoting Bacteria (PGPB), Arbuscular Mycorrhizal Fungi (AMF) Solutions.

	EC (mS/cm)		pH	
	Replication 1	Replication 2	Replication 1	Replication 2
Control	0.82±0.04	0.99±0.08	5.84±0.04	5.83±0.01
PGPB	0.97±0.04	0.99±0.08	5.91±0.15	5.84±0.02
AMF	1.03±0.07	1.09±0.04	5.94±0.18	5.86±0.03

Table 2.3. Means (±SD) of Electrical Conductivity (EC) and pH of Fertilizer Treatments.

	EC (mS/cm)		pH	
	Replication 1	Replication 2	Replication 1	Replication 2
Chemical	1.60±0.06	1.51±0.15	5.85±0.10	5.85±0.02
Food waste	2.02±0.08	2.13±0.21	5.89±0.12	5.87±0.03
Organic	1.33±0.10	1.36±0.14	5.84±0.05	5.87±0.03

Table 2.4. Results from Two-factor Analysis of Variance (P Values Are Listed) for the Effects of Fertilizers (F), Microorganisms (M), or Their Interaction on Plant Growth Parameters of Lettuce and Tomato Seedlings.

	Lettuce			Tomato		
Factor	F	M	F×M	F	M	F×M
Plant diameter	<0.001	0.587	0.504	–	–	–
Stem length	–	–	–	<0.001	0.701	0.667
Stem diameter	–	–	–	0.001	0.857	0.935
Leaf number	0.001	0.939	0.809	<0.001	0.318	0.606
Leaf length	<0.001	0.512	0.329	<0.001	0.201	0.997
Leaf width	<0.001	0.553	0.550	<0.001	0.763	0.319
Shoot fresh weight	<0.001	0.250	0.673	<0.001	0.343	0.719
Shoot dry weight	<0.001	0.105	0.499	<0.001	0.091	0.516
SPAD index	0.001	0.254	0.671	0.007	0.177	0.117

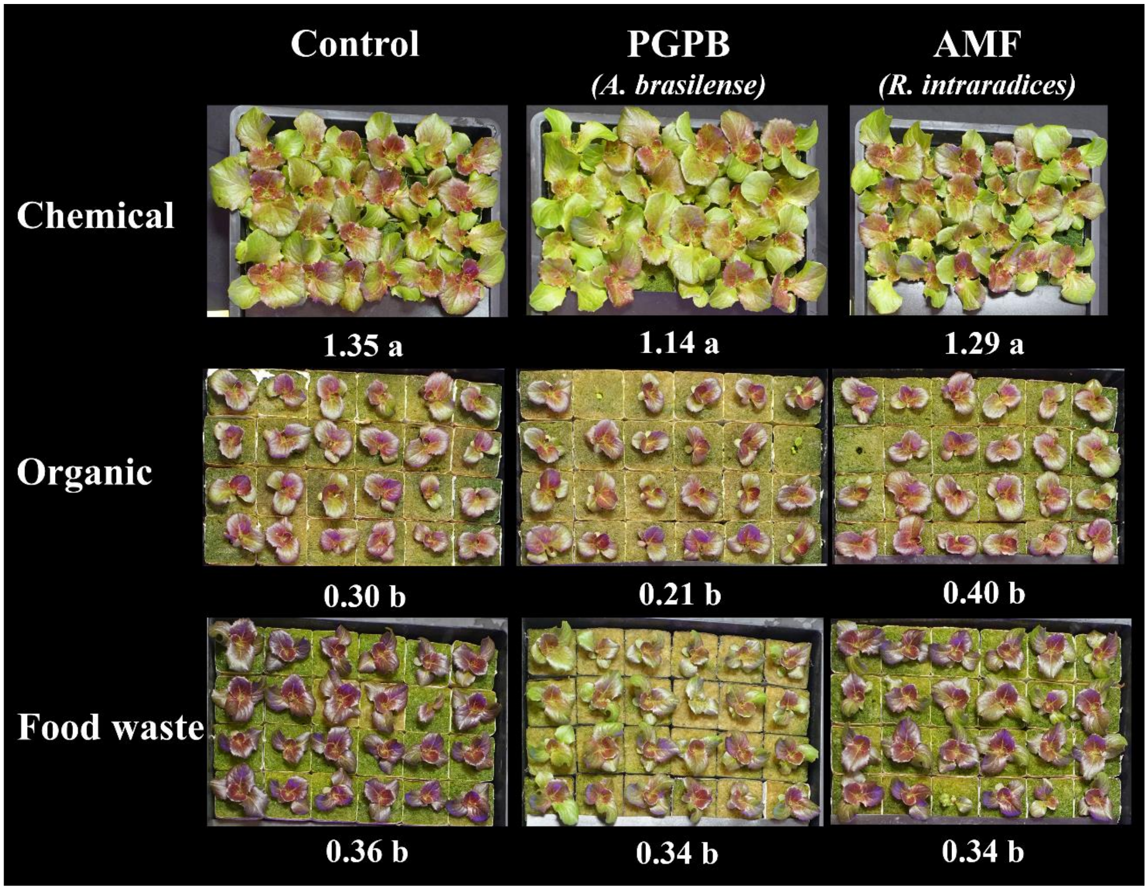

Figure 2.2. Fresh Weight (g) of Lettuce Seedlings Grown under Chemical, Organic, or Food Waste Fertilizer Without (Control) or with Plant Growth Promoting Bacteria (PGPB) or Arbuscular Mycorrhizal Fungi (AMF) Solutions Three Weeks after Treatments. Data Present the Mean of Two Replications with 10 Plants per Replication. Different Letters Are Statistically Different at $P < 0.05$.

Table 2.5. Plant Growth Characteristics of Lettuce Seedlings Grown under Chemical, Organic, or Food Waste Fertilizer Without (Control) or with Plant Growth Promoting Bacteria (PGPB) or Arbuscular Mycorrhizal Fungi (AMF) Solutions Three Weeks after Treatments. Data Present the Mean of Two Replications. Different Letters Are Statistically Different at $P<0.05$.

	Plant diameter (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	SPAD index	Shoot dry weight (g)
Chemical						
Control	6.5 a	4.7 a	4.9 a	4.1 a	24.4 c	0.08 a
PGPB	6.4 a	4.4 a	4.9 a	3.9 a	23.4 bc	0.07 a
AMF	6.5 a	4.5 a	5.1 a	4.0 a	25.4 bc	0.08 a
Organic						
Control	3.5 b	3.1 a	2.7 b	2.2 b	28.2 abc	0.03 b
PGPB	2.9 b	2.8 a	2.3 b	1.9 b	26.5 abc	0.02 b
AMF	3.9 b	3.2 a	3.1 b	2.5 b	26.7 abc	0.03 b
Food waste						
Control	3.6 b	3.2 a	2.7 b	2.1 b	32.1 abc	0.03 b
PGPB	3.7 b	3.5 a	3.0 b	2.2 b	30.5 ab	0.03 b
AMF	3.5 b	3.1 a	2.7 b	2.0 b	34.4 a	0.03 b

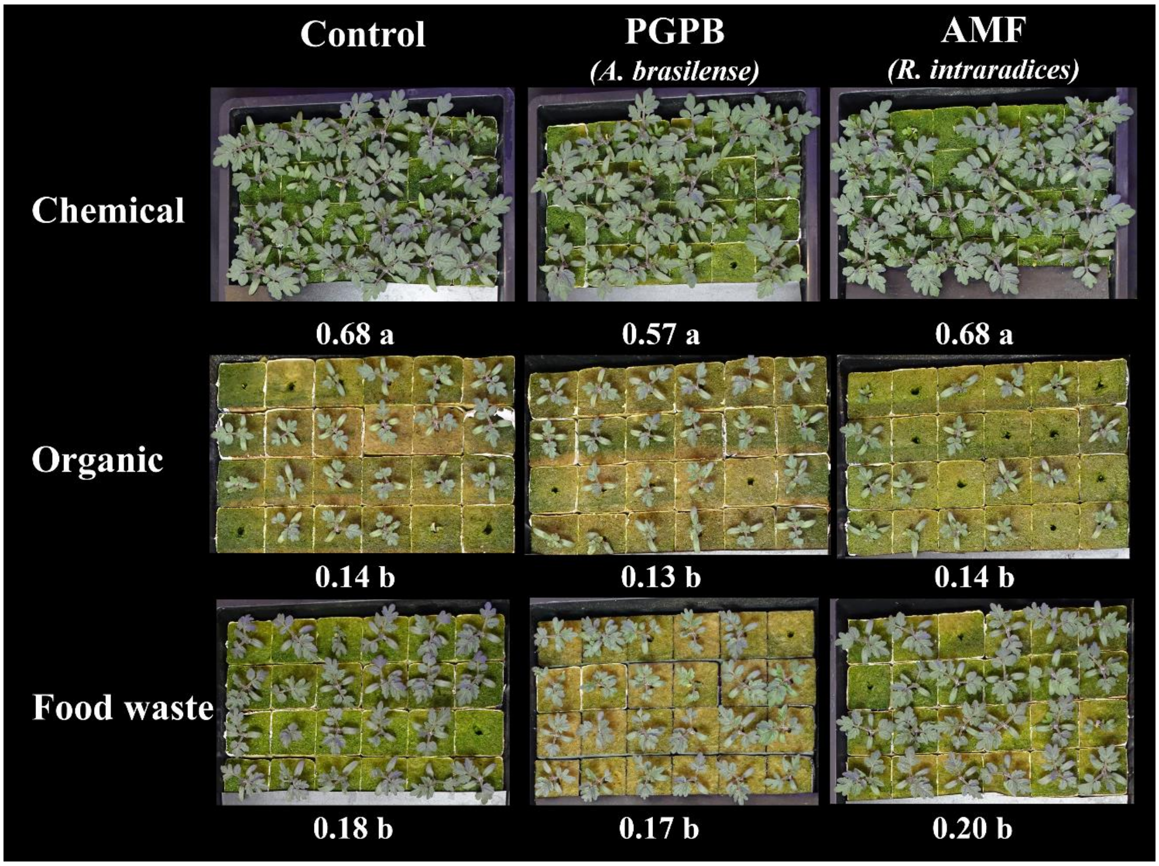

Figure 2.3. Fresh Weight (g) of Tomato Seedlings Grown under Chemical, Organic, or Food Waste Fertilizer Without (Control) or with Plant Growth Promoting Bacteria (PGPB) or Arbuscular Mycorrhizal Fungi (AMF) Solutions Three Weeks after Treatments. Data Present the Mean of Two Replications with 10 Plants per Replication. Different Letters Are Statistically Different at $P < 0.05$.

Table 2.6. Plant Growth Characteristics of Tomato Seedlings Grown under Chemical, Organic, or Food Waste Fertilizer Without (Control) or with Plant Growth Promoting Bacteria (PGPB) or Arbuscular Mycorrhizal Fungi (AMF) Solutions Three Weeks after Treatments. Different Letters Are Statistically Different at *P<0.05*.

	Stem diameter (cm)	Stem length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	SPAD index	Shoot dry weight (g)
Chemical							
Control	2.5 a	3.5 a	3.0 a	4.3 a	3.5 a	53.2 a	0.07 a
PGPB	2.3 ab	3.3 ab	3.0 a	3.9 ab	3.2 ab	52.5 a	0.05 a
AMF	2.5 a	3.7 a	3.2 a	4.3 a	3.7 a	50.8 a	0.07 a
Organic							
Control	1.5 b	2.1 c	2.1 b	2.0 c	1.4 c	41.8 a	0.02 b
PGPB	1.5 b	1.8 c	2.0 b	1.8 c	1.3 c	43.1 a	0.02 b
AMF	1.5 ab	2.0 c	2.0 b	2.2 c	1.6 c	45.1 a	0.02 b
Food Waste							
Control	1.6 ab	2.2 b	2.0 b	2.5 c	1.8 bc	42.1 a	0.02 b
PGPB	1.6 ab	2.4 bc	2.2 b	2.3 c	2.4 abc	52.5 a	0.02 b
AMF	1.6 ab	2.2 bc	2.3 b	2.6 bc	1.9 bc	53.8 a	0.02 b

REFERENCES

Agehara, S., & Warncke, D. D. (2005). Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. *Soil Science Society of America Journal*, *69*(6), 1844-1855.

Aulakh, M. S., Wassmann, R., Bueno, C., Kreuzwieser, J., & Rennenberg, H. (2001). Characterization of root exudates at different growth stages of ten rice (Oryza sativa L.) cultivars. *Plant biology*, *3*(02), 139-148.

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, cell & environment*, *32*(6), 666-681.

Balkrishna, A., Sharma, I. P., Arya, V., & Sharma, A. K. (2022). Biologicals and their plant stress tolerance ability. *Symbiosis*, *86*(3), 243-259.

Bartelme, R. P., Oyserman, B. O., Blom, J. E., Sepulveda-Villet, O. J., & Newton, R. J. (2018). Stripping away the soil: plant growth promoting microbiology opportunities in aquaponics. *Frontiers in microbiology, 9,* 8.

Batista, B. D., & Singh, B. K. (2021). Realities and hopes in the application of microbial tools in agriculture. Microbial Biotechnology, 14(4), 1258-1268.

Bashan, Y., & De-Bashan, L. E. (2002). Protection of tomato seedlings against infection by Pseudomonas syringae pv. tomato by using the plant growth-promoting bacterium *Azospirillum brasilense*. *Applied and environmental microbiology*, *68*(6), 2637-2643.

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., ... & Zhang, L. (2021). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science, 10,* 1068.

Bergstrand, K. J. (2022). Organic fertilizers in greenhouse production systems–a review. *Scientia Horticulturae, 295,* 110855.

Bi, G., Evans, W. B., Spiers, J. M., & Witcher, A. L. (2010). Effects of organic and inorganic fertilizers on marigold growth and flowering. *HortScience*, *45*(9), 1373-1377.

Buzby, J. C., Farah-Wells, H., & Hyman, J. (2014). The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. *USDA-ERS Economic Information Bulletin*, (121).

Chen, J. H. (2006, October). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In *International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use* (Vol. 16, No. 20, pp. 1-11). Land Development Department Bangkok Thailand.

Chojnacka, K., Moustakas, K., & Witek-Krowiak, A. (2020). Bio-based fertilizers: A practical approach towards circular economy. *Bioresource Technology*, *295*, 122223.

Costa, A. G., Bertolucci, S. K. V., Chagas, J. H., Ferraz, E. O., & Pinto, J. E. B. P. (2013). Biomass production, yield and chemical composition of peppermint essential oil using different organic fertilizer sources. *Ciência e Agrotecnologia*, *37*, 202-210.

Curtin, D., Beare, M. H., & Hernandez-Ramirez, G. (2012). Temperature and moisture effects on microbial biomass and soil organic matter mineralization. *Soil Science Society of America Journal*, *76*(6), 2055-2067.

Dasgan, H. Y., & Bozkoylu, A. (2006). Comparison of organic and synthetic-inorganic nutrition of soilless grown summer squash. In *VIII International Symposium on Protected Cultivation in Mild Winter Climates: Advances in Soil and Soilless Cultivation under 747* (pp. 523-528).

de Haas, B. R., Van Gerrewey, T., Perneel, M., Van Labeke, M. C., & Geelen, D. (2021, August). Beneficial microbes for improving circularity and yield in hydroponic crop cultivation. In *II International Symposium on Growing Media, Soilless Cultivation, and Compost Utilization in Horticulture 1317* (pp. 149-156).

Fichtner, K., & Schulze, E. D. (1992). The effect of nitrogen nutrition on growth and biomass partitioning of annual plants originating from habitats of different nitrogen availability. *Oecologia*, *92*(2), 236-241.

Franzluebbers, A. J. (1999). Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. *Applied Soil Ecology*, *11*(1), 91-101.4

Gaskell, M., & Smith, R. (2007). Nitrogen sources for organic vegetable crops. *HortTechnology*, *17*(4), 431-441.

Grobelak, A., Napora, A., & Kacprzak, M. (2015). Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecological Engineering*, *84*, 22-28.

Grunert, O., Hernandez-Sanabria, E., Vilchez-Vargas, R., Jauregui, R., Pieper, D. H., Perneel, M., ... & Boon, N. (2016). Mineral and organic growing media have distinct community structure, stability, and functionality in soilless culture systems. *Scientific reports*, *6*(1), 1-14.

Hayat, S., Faraz, A., & Faizan, M. (2017). Root exudates: composition and impact on plant–microbe interaction. *Biofilms in Plant and Soil Health. John Wiley & Sons Ltd*, 179-193.

Hernaández-Esquivel, A. A., Castro-Mercado, E., Valencia-Cantero, E., Alexandre, G., & García-Pineda, E. (2020). Application of *Azospirillum brasilense* lipopolysaccharides to promote early wheat plant growth and analysis of related biochemical responses. *Frontiers in Sustainable Food Systems*, *4,* 579976.

Jones, R. D., & Hood, M. A. (1980). Effects of temperature, pH, salinity, and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. *Microbial ecology*, *6*(4), 339-347.

Jurgilevich, A., Birge, T., Kentala-Lehtonen, J., Korhonen-Kurki, K., Pietikäinen, J., Saikku, L., & Schösler, H. (2016). Transition towards circular economy in the food system. *Sustainability, 8*(1), 69.

Kano, K., Kitazawa, H., Suzuki, K., Widiastuti, A., Odani, H., Zhou, S., ... & Sato, T. (2021). Effects of organic fertilizer on bok choy growth and quality in hydroponic cultures. *Agronomy*, *11*(3), 491.

Kang, S. M., Shaffique, S., Kim, L. R., Kwon, E. H., Kim, S. H., Lee, Y. H., ... & Lee, I. J. (2021). Effects of organic fertilizer mixed with food waste dry powder on the growth of Chinese cabbage seedlings. *Environments*, *8*(8), 86.

Khanghahi, M. Y., Ricciuti, P., Allegretta, I., Terzano, R., & Crecchio, C. (2018). Solubilization of insoluble zinc compounds by zinc solubilizing bacteria (ZSB) and optimization of their growth conditions. *Environmental Science and Pollution Research*, *25*(26), 25862-25868.

Khare, E., Chopra, J., & Arora, N. K. (2014). Screening for mcl-PHA-producing fluorescent Pseudomonads and comparison of mcl-PHA production under iso-osmotic conditions induced by PEG and NaCl. *Current microbiology*, *68*(4), 457-462.

Kim, S. J., Eo, J. K., Lee, E. H., Park, H., & Eom, A. H. (2017). Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology*, *45*(1), 20-24.

Kurt, D., & Ayan, A. K. (2014). Effect of the different organic fertilizer sources and doses on yield in organic tobacco (*Nicotiana tabacum* L.) production.

Li, S. X., Wang, Z. H., & Stewart, B. A. (2013). Responses of crop plants to ammonium and nitrate N. *Advances in agronomy*, *118*, 205-397.

Li, T., Lin, G., Zhang, X., Chen, Y., Zhang, S., & Chen, B. (2014). Relative importance of an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) and root hairs in plant drought tolerance. *Mycorrhiza*, *24*(8), 595-602.

Linn, D. M., & Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, *48*(6), 1267-1272.

Lopes, M. J. D. S., Dias-Filho, M. B., & Gurgel, E. S. C. (2021). Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Frontiers in Sustainable Food Systems*, *5*, 606454.

Lopez-Bucio, J., De la Vega, O. M., Guevara-Garcia, A., & Herrera-Estrella, L. (2000). Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. *Nature biotechnology*, *18*(4), 450-453.

Lucas García, J. A., Barbas, C., Probanza, A., Barrientos, M. L., & Gutierrez Mañero, F. J. (2001). Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *12*(5), 305-311.

Macintyre, O. J., Trevors, J. T., Dixon, M. A., & Cottenie, K. (2011). Application of plant growth promoting rhizobacteria in a hydroponics system for advanced life support in space. *Acta horticulturae,* (893), 1285-1292.

Majeed, A., Abbasi, M. K., Hameed, S., Yasmin, S., Hanif, M. K., Naqqash, T., & Imran, A. (2018). *Pseudomonas* sp. AF-54 containing multiple plant beneficial traits acts as growth enhancer of Helianthus annuus L. under reduced fertilizer input. *Microbiological research*, *216*, 56-69.

Mangmang, J. S., Deaker, R., & Rogers, G. (2015). Optimal plant growth-promoting concentration of *Azospirillum brasilense* inoculated to cucumber, lettuce and tomato seeds varies between bacterial strains. *Israel Journal of Plant Sciences*, *62*(3), 145-152.

Mickan, B. S., Ren, A. T., Buhlmann, C. H., Ghadouani, A., Solaiman, Z. M., Jenkins, S., ... & Ryan, M. H. (2022). Closing the circle for urban food waste anaerobic digestion: The use of digestate and biochar on plant growth in potting soil. *Journal of Cleaner Production*, *347*, 131071.

Mikiciuk, G., Sas-Paszt, L., Mikiciuk, M., Derkowska, E., Trzciński, P., Głuszek, S., ... & Rudnicka, J. (2019). Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and rhizosphere bacteria. *Mycorrhiza*, *29*(5), 489-501.

Mishra, N., & Sundari, S. K. (2013). Native PGPMs as bioinoculants to promote plant growth: response to PGPM inoculation in principal grain and pulse crops. *International Journal of Agriculture Food Science & Technology*, *4*(10), 1055-1064.

Moncada, A., Vetrano, F., & Miceli, A. (2020). Alleviation of salt stress by plant growth-promoting bacteria in hydroponic leaf lettuce. *Agronomy*, *10*(10), 1523.

Moncada, A., Miceli, A., & Vetrano, F. (2021). Use of plant growth-promoting rhizobacteria (PGPR) and organic fertilization for soilless cultivation of basil. *Scientia Horticulturae*, *275*, 109733.

Neumann, G., Bott, S., Ohler, M. A., Mock, H. P., Lippmann, R., Grosch, R., & Smalla, K. (2014). Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as affected by different soils. *Frontiers in microbiology*, *5*, 2.

Nosheen, S., Ajmal, I., & Song, Y. (2021). Microbes as biofertilizers, a potential approach for sustainable crop production. *Sustainability*, *13*(4), 1868.

Oelkers, E. H., & Valsami-Jones, E. (2008). Phosphate mineral reactivity and global sustainability. *Elements*, *4*(2), 83-87.

Oljira, A. M., Hussain, T., Waghmode, T. R., Zhao, H., Sun, H., Liu, X., ... & Liu, B. (2020). Trichoderma enhances net photosynthesis, water use efficiency, and growth of wheat (Triticum aestivum L.) under salt stress. *Microorganisms*, *8*(10), 1565.

Ortíz-Castro, R., Contreras-Cornejo, H. A., Macías-Rodríguez, L., & López-Bucio, J. (2009). The role of microbial signals in plant growth and development. *Plant signaling & behavior*, *4*(8), 701-712.

Paradiso, R., Arena, C., De Micco, V., Giordano, M., Aronne, G., & De Pascale, S. (2017). Changes in leaf anatomical traits enhanced photosynthetic activity of soybean grown in hydroponics with plant growth-promoting microorganisms. *Frontiers in Plant Science*, *8*, 674.

Pelayo Lind, O., Hultberg, M., Bergstrand, K. J., Larsson-Jönsson, H., Caspersen, S., & Asp, H. (2021). Biogas digestate in vegetable hydroponic production: pH dynamics and pH management by controlled nitrification. *Waste and Biomass Valorization*, *12*(1), 123-133.

Phibunwatthanawong, T., & Riddech, N. (2019). Liquid organic fertilizer production for growing vegetables under hydroponic condition. *International Journal of Recycling of Organic Waste in Agriculture*, *8*(4), 369-380.

Rudrappa, T., Czymmek, K. J., Paré, P. W., & Bais, H. P. (2008). Root-secreted malic acid recruits beneficial soil bacteria. *Plant physiology*, *148*(3), 1547-1556.

Saijai, S., Ando, A., Inukai, R., Shinohara, M., & Ogawa, J. (2016). Analysis of microbial community and nitrogen transition with enriched nitrifying soil microbes for organic hydroponics. *Bioscience, biotechnology, and biochemistry*, *80*(11), 2247-2254.

Savci, S. (2012). Investigation of effect of chemical fertilizers on environment. *Apcbee Procedia*, *1*, 287-292.

Sheridan, C., Depuydt, P., De Ro, M., Petit, C., Van Gysegem, E., Delaere, P., ... & Geelen, D. (2017). Microbial community dynamics and response to plant growth-promoting microorganisms in the rhizosphere of four common food crops cultivated in hydroponics. *Microbial ecology*, *73*(2), 378-393.

Shinohara, M., Aoyama, C., Fujiwara, K., Watanabe, A., Ohmori, H., Uehara, Y., & Takano, M. (2011). Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. *Soil science and plant nutrition*, *57*(2), 190-203.

Silgram, M., & Shepherd, M. A. (1999). The effects of cultivation on soil nitrogen mineralization. *Advances in agronomy*, *65*, 267-311.

Smith, S. R., & Hadley, P. (1989). A comparison of organic and inorganic nitrogen fertilizers: Their nitrate-N and ammonium-N release characteristics and effects on the growth response of lettuce (*Lactuca sativa* L. cv. Fortune). *Plant and soil*, *115*(1), 135-144.

Souza, R. D., Ambrosini, A., & Passaglia, L. M. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and molecular biology*, *38*, 401-419.

Stoknes, K., Wojciechowska, E., Jasińska, A., Gulliksen, A., & Tesfamichael, A. A. (2017). Growing vegetables in the circular economy; cultivation of tomatoes on green waste compost and food waste digestate. In *International Symposium on Greener Cities for More Efficient Ecosystem Services in a Climate Changing World 1215* (pp. 389-396).

Stoknes, K., Scholwin, F., Jasinska, A., Wojciechowska, E., Mleczek, M., Hanc, A., & Niedzielski, P. (2019). Cadmium mobility in a circular food-to-waste-to-food system and the use of a cultivated mushroom (*Agaricus subrufescens*) as a remediation agent. *Journal of environmental management*, *245*, 48-54.

Tesfaye, M., Dufault, N. S., Dornbusch, M. R., Allan, D. L., Vance, C. P., & Samac, D. A. (2003). Influence of enhanced malate dehydrogenase expression by alfalfa on diversity of rhizobacteria and soil nutrient availability. *Soil Biology and Biochemistry*, *35*(8), 1103-1113.

Watts-Williams, S. J., Turney, T. W., Patti, A. F., & Cavagnaro, T. R. (2014). Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas. *Plant and Soil*, *376*(1), 165-175.

Zahradník, A., & Petrikova, K. (2007). Effect of alternative organic fertilizers on the nutritional value and yield of head cabbage. *Hort. Sci.(Prague)*, *34*(2), 65-71.

CHAPTER 3

INVESTIGATING THE EFFECTS OF APPLICATION FREQUENCIES OF PLANT GROWTH-PROMOTING MICROORGANISMS' EFFECT ON LETTUCE SEEDLINGS

ABSTRACT

Many studies have sought to understand how plant growth-promoting microorganisms (PGPMs) can be used to improve plant growth in both soil and soilless cultivation. However, PGPM inoculation methods have yet to be fully developed for soilless crop production in controlled environments. In this study, we investigated how the application frequency of arbuscular mycorrhizal fungi (AMF), plant growth promoting bacteria (PGPB), and combination of AMF+PGPB influence seedling growth in lettuce (*Lactuca sativa*) cultivars 'Cherokee' and 'Rex'. Lettuce seeds were sown in rockwool plugs and inoculated with *Azospirillium brasilense* (1.0×10^8 CFU/L), *Rhizophagus intraradices* (250 propagules/L), or a combination of inoculum were applied to the rootzone via sub-irrigation at sowing and every two or seven days afterwards. Lettuce seedlings were fertilized at 100 ppm total nitrogen with an organic fertilizer derived from food waste and grown in an indoor vertical farm at 22 °C under sole-source LED lighting with an 18-h photoperiod at a photosynthetic photon flux density of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Three weeks after seed sow, a 2-day frequency of AMF application increased shoot fresh weight of lettuce 'Cherokee' by 58% and 'Rex' by 51% compared to un-inoculated control. In contrast, PGPB application, regardless of frequency, and a 7-day frequency application of AMF without or with PGPB had little effects on lettuce seedling growth in both cultivars. These results suggest that increasing the application frequency can enhance the plant growth promoting effects of some PGPMs, such as AMF.

Keywords: *Azosprillium*, beneficial microbes, controlled environment, inoculation methods, plant growth-promoting microbes, *Rhizophagus*

Abbreviations:

AD, Anerobic Digestate; AMF, Arbuscular Mycorrhizal Fungi; ANOVA, Analysis of Variance; DLI, Daily Light Integral; Dw, Shoot Dry Weight; EC, Electrical Conductivity; FW, Shoot Fresh Weight; K, Potassium; LEDs, Light Emitting Diodes; N, Nitrogen; P, Phosphorus; PGPB, Plant Growth Promoting Bacteria; PGPMs, Plant Growth Promoting Microorganisms; PPFD, Photosynthetic Photon Flux Density; SPAD, Soil-plant Analysis Development

Introduction

A fertilizer is any kind of material, either be naturally or synthetically made that supplies plants with essential nutrients for plant growth and maximum yield (Hati and Bandyoopadhay, 2011). Some fertilizers can be organic that come from either plant or animal origin, whereas inorganic fertilizers are compounds that are mined from mineral deposits or manufactured from chemical processes (Gupta and Hussain, 2014; Jastrzębska et al., 2022). Inorganic fertilizers are commonly used for crop production because they have high nutrient concentration, easily dissolve in water, and provide readily available nutrients to plants (Jastrzębska et al., 2022). However, using inorganic fertilizers has detrimental effects on the environment by mining activities, greenhouse gas emissions, and in-ground water contamination (Jastrzębska et al., 2022). In addition, as recent inorganic fertilizer shortage raises fears of a global food crisis (Brunelle et al., 2015), there is a growing demand for organic fertilizers, such as manure and anaerobic digestates. Yet organic fertilizers have lower nutrient content and nutrient release rate, so they have been less effective in meeting crop nutrient needs (Connor, 2008).

Using plant growth promoting microorganisms (PGPMs) has been suggested as one way to improve the efficacy of organic fertilizers. PGPMs are symbiotic rhizosphere dwelling microorganisms that can colonize plant roots and provide benefits to their hosts by means of phytohormones production, increased nutrient uptake, pathogen resistance, and environmental stresses (Lopes et al., 2021). Commonly used PGPMs for crop production include arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacterial (PGPB) communities. Under organic fertilization, PGPMs can facilitate mineralization process of the organic fertilizer, and thus nutrient availability and uptake (Kumar et al., 2018).

However, the effects of PGPMs on plant growth depend on successful inoculation of PGPMs and the interaction between PGPMs and plants which are influenced by co-inoculation, inoculum density, inoculation method, and microbial competition (Hernández-Montiel et al., 2017; Msimbira and Smith, 2020; Venturi and Keel, 2016). For example, Meena et al. (2010) demonstrated co-inoculation AMF *Piriformospora indica* in combination of a PGPB *Pseudomonas striata* promoted increases shoot dry weight of chickpea (*Cicer arietinum*) by 22-50% compared to single species inoculation and uninoculated controls. Similarly, a greenhouse study by Begum et al. (2021) have shown co-inoculation of AMF *Glomus versiforme* with PGPB *Bacillus methyloptrophicus* promoted increased shoot fresh weight of *Nicotiana tabacum* L. by 21-66% compared to single species or control plants. In rapeseed (*Brassica napus*), PGPM *Phyllobacterium brassicaearum* showed plant growth promoting effects in a dose-dependent manner with the maximum growth potential on roots from bacterial densities of around 10^8 CFU/mL.

In our previous study, the inoculation of AMF *Rhizophagus intraradices* and PGPB *Azospirillium brasilense* didn't influence plant growth in both lettuce and tomato seedlings regardless of fertilizer types (Chapter 2). Given that we used single species inoculation of AMF or PGPB and applied them weekly, we assumed that little effects of AMF and PGPB on plant growth could be attributed to insufficient method for inoculation. The objective of this study was to investigate how inoculation frequencies and co-inoculation of PGPMs influence their plant growth promoting effects on lettuce seedlings. We postulated that (1) a higher frequency application of PGPM would improve PGPM colonization with host plant and promote plant growth and (2) co-inoculation of AMF *Rhizophagus intraradices* and PGPB *Azospirillium brasilense* will promote symbiotic interactions between the two species and host and subsequently, plant growth.

Materials and Methods

Seed inoculation and propagation

Butterhead lettuce 'Rex' and red leaf lettuce 'Cherokee' (Johnny's Selected Seeds, Winslow, ME) were selected for this study. On November 1st, 2021 (rep. 1), and

March 16th, 2022 (rep. 2), four PGPM solutions were made to inoculate seeds and rockwool medium before sowing seeds: AMF, PGPB, mixture of AMF and PGPB inoculants (Mix), and tap water without inoculants (Control). The AMF solution was made to provide 0.57 propagules/mL of *Rhizophagus intraradices* by hand mixing 44 g of mycorrhizal wettable powder (Mykos; Reforestation Technologies International; Gilroy, CA) into 19 L of tap water. The PGPB solution was made to provide 5×10^8 CFU/mL of *Azosprillium brasilense* by mixing 100 mL of liquid bacterial inoculant (Azos ® red liquid plant growth promoting bacteria; Reforestation Technologies International; Gilroy, CA) into 19 L of tap water. The Mix solution was made by adding 44 g of mycorrhizal wettable powder (Mykos; Reforestation Technologies International) and 100 mL of liquid bacterial inoculant (Azos ® red liquid plant growth promoting bacteria; Reforestation Technologies International) to 19 liters of tap water. The pH of all microorganism solutions was adjusted to 5.8-6.0 with 50% sulfuric acid. The pH and EC of PGPM solutions were measured using a handheld pH and EC meter (HI9814; Hannah Instruments; Woonsocket, RI).

Eighty seeds of each lettuce cultivar were soaked for one hour in 10 mL of the AMF, PGPB, Mix, or Control solution in 50 mL glass beakers following Mangmang et al. (2014). The 200-cell (2.5 cm × 2.5 cm) rockwool plugs (AO 25/40 Starter Plugs; Grodan, Milton, ON, Canada) were cut into 5 sections, each with 40 cells, and each section was soaked with one of the PGPM solutions for 20 mins. Inoculated rockwool plugs were then placed in plant trays (20.8 cm × 10.8 cm × 2.4 cm; HGC726298 Super Sprouter Quad Thick Tray 10 × 20; Hawthorne, Vancouver, WA), and the inoculated seeds were sown into the inoculated rockwool. The seeded rockwool plugs were covered with humidity domes and placed on one growing rack with three tiers inside the temperature controlled environmental research vertical farm (Arizona State University Polytechnic campus, Mesa, AZ). The humidity domes were removed three days after sowing when the seeds of both lettuce cultivars were fully germinated.

Seedling inoculation

After germination, the remaining initial PGPM solutions were discarded, and the lettuce seedlings were sub-irrigated every two (2-day frequency) or seven days (7-day frequency) with food waste fertilizer (0.06-0.026-0.1191 Climate Saver, Homer Farms Inc.) at a 100 ppm N without (Control) or with PGPM inoculants. During the seedling stage, PGPM inoculants provided 0.57 propagules/mL of *Rhizophagus intraradices* for AMF treatments, 1×10^8 CFU/mL of *Azospirillium brasilense* for PGPB treatments, and 0.57 propagules/mL of *Rhizophagus intraradices* + 1×10^8 CFU/mL of *Azospirillium brasilense* for Mix treatments. All food waste fertilizer + PGPM solutions maintains the pH of 5.8 by using potassium bicarbonate (Earthborn Elements; American Fork, UT) and/or 50% sulfuric acid (Tables 3.1). The pH and EC of PGPM solutions were monitored with a handheld pH and EC meter (HI9814; Hannah Instruments). For 7-day frequency PGPM treatments, seedlings were sub-irrigated every two or three days with food waste fertilizer at a 100 ppm without PGPM inoculants.

Growth conditions

During the experiment period, air temperature was set to 22 °C and was monitored at each tier of growing rack and recorded once every 15 minutes by a thermometer hydrometer (H57075; Shenzhen Intellirocks Tech Co., Shenzhen, Guangdong, China) (Table 3.2). Sole-source lighting was provided from blue + red + white LEDs (T8 Double-Row LED Indoor Grow Light; Homer Farms Inc., Mesa, AZ) at an average photosynthetic photon flux density (PPFD) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 18-hour photoperiod. The average PPFD was measured using a spectroradiometer (PS-300, StellerNet, Inc., Tampa, FL) at nine predetermined locations on the growing area at tray height.

Data collection and analysis

At transplant stage, which was 24 days after sowing, photographs of an average size plant from all treatments were taken under a white LED lamp to distinguish plant growth attributes. After the photos were taken, ten random plants from each treatment per cultivar were selected for data collection. For individual lettuce seedlings, we measured the plant diameter (with a ruler), leaf number (when >1.5 cm), SPAD index as an indicator for relative chlorophyll concentration per unit leaf area [with a handheld SPAD meter (SPAD-502; Konica Minolta Sensing, Inc., Chiyoda, Tokyo, Japan)], and shoot fresh and dry weights [on an analytical balance (PB602-S; Mettler Toledo, Columbus, OH)]. For shoot dry weight, the plants were drying for five days in a drying oven (Hafo 1600 Oven, VWR International, LLC, Aurora, CO) at 86°C. The statistical analysis was preformed using a two-factor ANOVA test using SAS statistical program.

This experiment was conducted as a randomized complete block design with replication (block), PGPM and frequency treatments (experimental unit), and plant per species (subsample). Data from two replications were pooled and analyzed them with SAS (version 9.4; SAS Institute, Inc., Cary, NC) using the PROC MIXED procedure [with two fixed factors for PGPM and application frequency treatments, two random factors of blocks and the interaction among blocks and treatments] that provided pairwise comparisons between treatments by using Tukey's honestly significant test at $P<0.05$.

Results

In both lettuce 'Cherokee' and 'Rex', seedlings grown with a 2-day frequency of AMF had 51-58% greater shoot fresh weight compared to control (Figure 3.2 and Tables 3.3 and 3.4). However, there was little to no effect from a 7-day frequency of AMF or application of PGPB alone or with AMF on plant diameter, leaf number, SPAD index, shoot dry weight.

Discussion

Inoculation density and frequency influence the successful microbial inoculations (Lopes et al., 2021; Hernández-Montiel et al., 2017). In this study, using AMF increased shoot fresh weight of lettuce 'Cherokee' and 'Rex' seedlings only with 2-day frequency but not with 7-day frequency compared to control. AMF are attracted to root exudates especially when radicals first develop, and hyphae can more easily penetrate thin-walled cells from terminal roots (Xie et al., 2020). However, because AMF are not very mobile within the rhizosphere, high density of AMF increases the chance for the propagules to develop successful colonies on the developing plant roots (Franson and Bethlenfalvay, 1989). Once AMF develop colonies in plant roots, AMF facilitate host plant growth by increasing root surface area via hyphae formation increasing nutrient and water uptake, phosphate allocation, and increasing nutrient availability by decomposing organic matter (Birhane et al., 2012; Bowles et al., 2016; Rouphael et al., 2015; Paterson et al., 2016). The increases in plant growth with AMF 2-day frequency in our study is therefore likely from the increased successful AMF colonization with the increased density of AMF propagules near rootzone.

In contrast to AMF applications, the PGPB treatment had no effect on plant growth parameters in two lettuce cultivars regardless of application frequency. *Azospirillum*, as one of the most commonly and popularly used PGPB genera, have shown plant growth promoting effects in a wide range of vegetable species (Bashan and de-Bashan, 2010). In lettuce, several studies investigated the impacts of *Azospirillum brasilense* inoculation, but plant responses varied. For example, in lettuce 'Salina', inoculation of *Azospirillum brasilense* increased seedling growth (Mangmang et al., 2014). In lettuce 'Elisa', seed inoculation of *Azospirillum brasilense* increased photosynthesis and plant growth with salt stress but not without salt stress (Fasciglione et al., 2015). Given that growth promoting ability of some bacteria can be highly specific to certain cultivar and depend on plant growing conditions (Mangmang et al., 2015; Katiyar et al., 2016), little effects of *Azospirillum brasilense* on lettuce growth in this study could be attributed to cultivar specific responses or/and a relatively ideal plant growing environment.

In addition, little effects from PGPB in this study could be a consequence of less favorable environmental conditions, such as temperature, pH, and moisture content, for *Azospirillum brasilense*. Temperature ranges can affect microbial growth by dictating the function of proteins within their cells (Sargen, 2020). Specifically, for PGPB, 30°C -37°C has been reported to be most optimum for *Azospirillum brasilense* (Romero-Perdomo et al., 2015; Tarrand et al., 1978). pH has been recognized to strongly influence microbial growth (Ratzke and Gore, 2018). *Azospirillum brasilense*grow vigorously at a pH between 6.8 and 7 (Bocatti et al., 2022; Coughlan et al., 2000; Romero-Perdomo et al., 2015). Low water availability can inhibit PGPB activity by reduced hydration limiting the activity of microbial enzymes, while too much available water can lower oxygen levels in turn reducing microbial activity (Borowik and Wyszkowska, 2016; Stark and Firestone, 1995). In this study, as the air temperature of 22°C, nutrient solution pH of 5.8, and continuously wet rootzone were maintained for optimum lettuce growth, lower temperature, pH, and oxygen availability than the desired conditions for *Azospirillum brasilense* might contribute to the limited activity and plant growth promoting effects from *Azospirillum brasilense*.

Compared to single inoculation, co-inoculating plants with AMF and PGPB can have greater plant growth promoting effects associated with their beneficial interrelationship (Begum et al., 2022). For example, co-inoculation of *Rhizophagus* *irregularis* and *Bradyrhizobium spp*. increased shoot dry weight of *Acacia magnum* by 19-50% compared to single-species or un-inoculated controls (Weber et al., 2005). In cherry tomato (*Solanum lycopersicum* var. cerasiforme 'Camelia'), co-inoculation of *Azospirllium* and *Rhizophagus* increased overall plant biomass of by 15-33% compared to single-species inoculation or un-inoculated controls (Lira-Saldivar et al., 2014). In contrast, in this study, co-inoculation of AMF and PGPB treatments showed a relatively similar plant growth promoting effects with AMF in both lettuce and tomato seedlings. Considering that there were little effects from PGPB treatment itself, our results is likely mostly from AMF without synergetic effects between AMF and PGPB treatments. Similarly, co-inoculation of *Azospirllium* and *Rhizophagus* did not significantly increase plant growth or yield of soilless *Cucumis sativus* compared to a single-species inoculation or the un-inoculated controls (Abdelaziz and Pokluda, 2007).

Conclusion

This study shows that increasing inoculation frequency of AMF *Rhizophagus irregularis* from weekly to every two days increases plant growth of lettuce 'Cherokee' and 'Rex. However, inoculating PGPB *Azospirillum brasilense had* little effects on plant growth in two lettuce cultivars regardless of application frequency. With little effects from *Azospirillum brasilense,* co-inoculation with *Rhizophagus irregularis* showed a relatively similar plant responses with single inoculation of *Rhizophagus irregularis.* These results suggest the application frequency can influence the successful inoculation of PGPMs and their plant growth promoting effects for at least some PGPM species.

However, further research is needed to identify the optimum application methods and environmental conditions to improve the effectiveness of PGPMs uses.

Table 3.1. Means (±SD) of Electrical Conductivity (EC) and pH of Control, Arbuscular Mycorrhizal Fungi Solutions (AMF), Plant Growth Promoting Bacteria (PGPB) Solution, and AMF+PGPB (Mix) Solutions.

		EC (mS/cm)		pH	
		Replication 1	Replication 2	Replication 1	Replication 2
Control:	2-day	3.41±0.34	3.61±0.02	5.84±0.03	5.86±0.03
Control:	7-day	3.43±0.34	3.61±0.02	5.86±0.02	5.87±0.03
AMF:	2-day	3.47±0.38	3.60±0.01	5.87±0.02	5.87±0.02
AMF:	7-day	3.40±0.34	3.60±0.02	5.85±0.03	5.87±0.03
PGPB:	2-day	3.49±0.23	3.60±0.00	5.86±0.03	5.86±0.02
PGPB:	7-day	3.45±0.31	3.61±0.02	5.86±0.03	5.87±0.03
Mix:	2-day	3.46±0.24	3.62±0.04	5.87±0.02	5.87±0.02
Mix:	7-day	3.44±0.30	3.62±0.04	5.87±0.03	5.86±0.03

Table 3.2. Means (±SD) of Air Temperature (°C) and Relative Humidity (%) of Both Replications Inside of the Growing Rack.

Location inside of growing rack	Air Temperature (°C)		Relative Humidity (%)	
	Replication 1	Replication 2	Replication 1	Replication 2
Top	22.5±1.34	22.5±2.21	67.1±9.95	70.9±9.42
Middle	22.4±1.41	22.3±2.26	67.2±9.12	74.8±9.17
Bottom	22.4±1.34	22.2±2.24	68.8±9.31	75.6±9.80

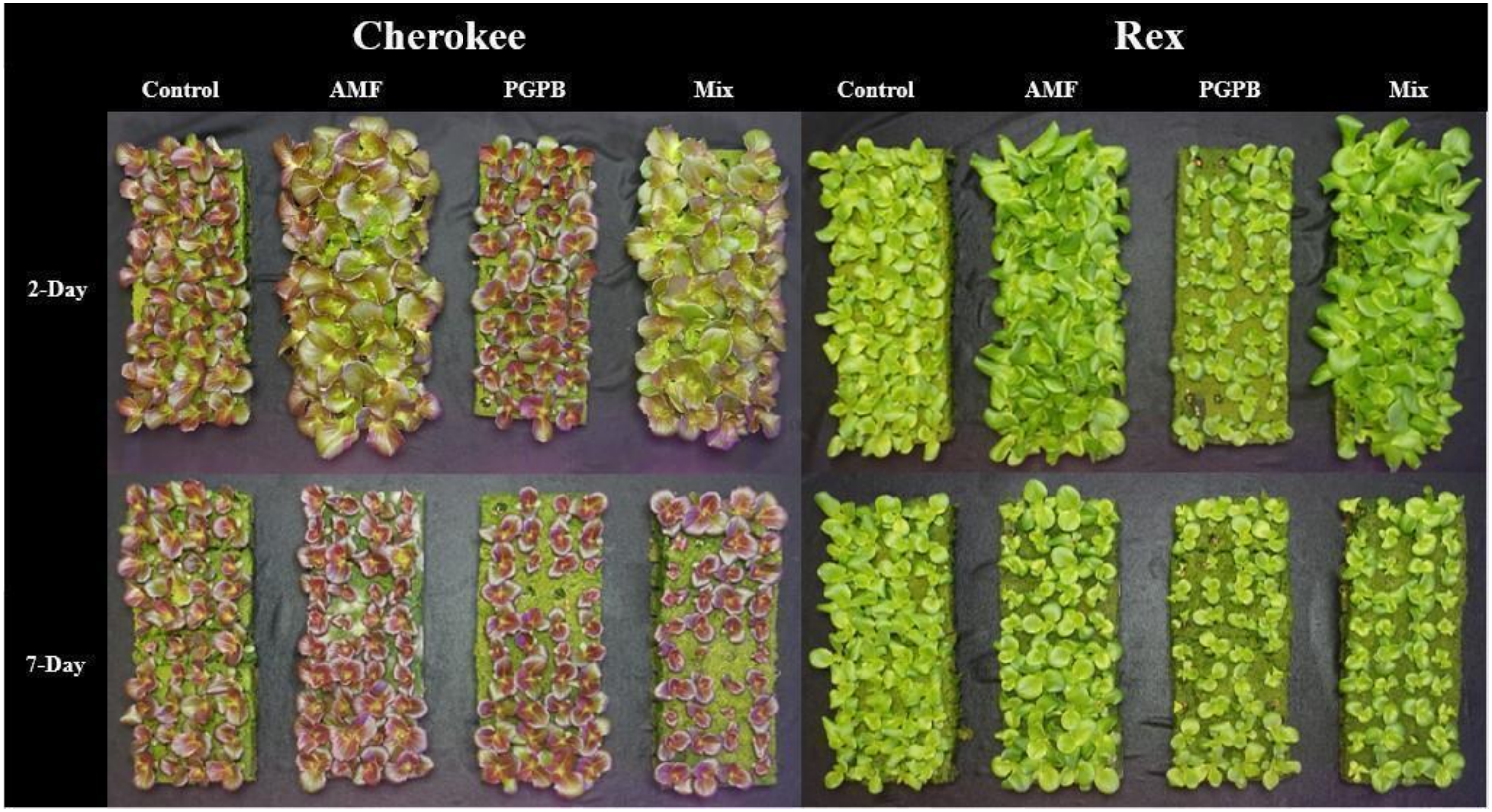

Figure 3.2. Lettuce Seedlings ‘Cherokee’ and ‘Rex’ Grown at 22 ºC Without (Control) or with Arbuscular Mycorrhizal Fungi (AMF), Plant Growth Promoting Bacteria (PGPB), or Mixture of AMF and PGPB (Mix) Solutions at 2-day or 7-day Application Frequency for 24 Days after Sowing.

Table 3.3. Growth Characteristics of Lettuce Seedlings 'Cherokee' Grown at 22 ºC Without (Control) or with Arbuscular Mycorrhizal Fungi (AMF), Plant Growth Promoting Bacteria (PGPB), or Mixture of AMF and PGPB (Mix) Solutions at 2-day or 7-day Application Frequency for 24 Days after Sowing. Data Present the Mean of Two Replications. Different Letters Are Statistically Different at *P < 0.05*.

	Plant diameter (cm)	leaf number	SPAD	Shoot FW (g)	Shoot DW (g)
2-day					
Control	4.3 a	5.3 a	28.3 c	0.63 b	0.04 ab
AMF	5.5 abc	5.9 a	29.7 bc	1.16 a	0.07 a
PGPB	4.2 abc	5.2 a	31.5 abc	0.60 b	0.04 ab
Mix	5.2 ab	5.6 a	29.1 c	0.85 ab	0.05 ab
7-day					
Control	3.4 c	4.7 a	32.3 abc	0.39 b	0.03 b
AMF	3.8 bc	5.0 a	34.4 a	0.46 b	0.04 ab
PGPB	3.6 bc	4.8 a	33.6 ab	0.40 b	0.03 b
Mix	3.9 bc	5.0 a	31.4 abc	0.50 b	0.04 ab
Significance					
Frequency (F)	0.001	0.008	0.001	0.001	0.009
Microorganism (M)	0.037	0.198	0.025	0.024	0.030
F×M	0.296	0.756	0.278	0.071	0.197

Table 3.4. Growth Characteristics of Lettuce Seedlings ‘Rex’ Grown at 22 °C Without (Control) or with Arbuscular Mycorrhizal Fungi (AMF), Plant Growth Promoting Bacteria (PGPB), or Mixture of AMF and PGPB (Mix) Solutions at 2-day or 7-day Application Frequency for 24 Days after Sowing. Data Present the Mean of Two Replications. Different Letters Are Statistically Different at $P < 0.05$.

	Plant diameter (cm)	leaf number	SPAD	Shoot FW (g)	Shoot DW (g)
2-day					
Control	3.9 b	6.6 ab	29.7 a	0.58 bc	0.04 ab
AMF	5.6 a	7.2 a	32.1 a	0.97 a	0.06 a
PGPB	3.3 b	5.4 b	31.9 a	0.39 c	0.03 b
Mix	5.3 a	7.0 a	30.1 a	0.92 ab	0.06 a
7-day					
Control	4.0 b	5.7 ab	33.1 a	0.46 c	0.03 ab
AMF	4.2 b	6.0 ab	33.4 a	0.52 c	0.04 ab
PGPB	3.7 b	5.7 ab	31.0 a	0.45 c	0.04 ab
Mix	3.8 b	6.0 ab	34.0 a	0.57 bc	0.05 ab
Significance					
Frequency (F)	0.003	0.008	0.038	0.002	0.069
Microorganism (M)	0.001	0.020	0.570	0.002	0.005
F×M	0.002	0.095	0.178	0.015	0.181

REFERENCES

Abdelaziz, M. E., & Pokluda, R. (2007, September). Response of cucumbers grown on two substrates in an open soilless system to inoculation with microorganisms. In *International Symposium on Growing Media 2007 819* (pp. 157-164).

Bashan, Y., & De-Bashan, L. E. (2010). How the plant growth-promoting bacterium Azospirillum promotes plant growth—a critical assessment. *Advances in agronomy*, *108*, 77-136.

Begum, N., Wang, L., Ahmad, H., Akhtar, K., Roy, R., Khan, M. I., & Zhao, T. (2021). Co-inoculation of arbuscular mycorrhizal fungi and the plant growth-promoting rhizobacteria improve growth and photosynthesis in tobacco under drought stress by up-regulating antioxidant and mineral nutrition metabolism. *Microbial Ecology*, *83*(4), 971-988.

Birhane, E., Sterck, F. J., Fetene, M., Bongers, F., & Kuyper, T. W. (2012). Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. *Oecologia*, *169*(4), 895-904.

Bocatti, C. R., Ferreira, E., Ribeiro, R. A., de Oliveira Chueire, L. M., Delamuta, J. R. M., Kobayashi, R. K. T., ... & Nogueira, M. A. (2022). Microbiological quality analysis of inoculants based on Bradyrhizobium spp. and Azospirillum brasilense produced “on farm” reveals high contamination with non-target microorganisms. *Brazilian Journal of Microbiology*, *53*(1), 267-280.

Borowik, A., & Wyszkowska, J. (2016). Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant, Soil and Environment*, *62*(6), 250-255.

Bowles, T. M., Barrios-Masias, F. H., Carlisle, E. A., Cavagnaro, T. R., & Jackson, L. E. (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. *Science of the Total Environment*, *566*, 1223-1234.

Brunelle, T., Dumas, P., Souty, F., Dorin, B., & Nadaud, F. (2015). Evaluating the impact of rising fertilizer prices on crop yields. *Agricultural economics*, *46*(5), 653-666.

Connor, D. J. (2008). Organic agriculture cannot feed the world. *Field Crops Research*, *106*(2), 187.

Coughlan, A. P., Dalpé, Y., Lapointe, L., & Piché, Y. (2000). Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Canadian Journal of Forest Research, 30*(10), 1543-1554.

Fasciglione, G., Casanovas, E. M., Quillehauquy, V., Yommi, A. K., Goñi, M. G., Roura, S. I., & Barassi, C. A. (2015). Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. *Scientia Horticulturae, 195*, 154-162.

Franson, R. L., & Bethlenfalvay, G. J. (1989). Infection Unit Method of Vesicular-Arbuscular Mycorrhizal Propagule Determination. *Soil Science Society of America Journal, 53*(3), 754-756.

Gupta, A. R. T. I., & Hussain, N. I. S. R. E. E. N. (2014). A critical study on the use, application and effectiveness of organic and inorganic fertilizers. *Journal of Industrial Pollution Control*, *30*(2), 191-194.

Hati, K., & Bandyoopadhay, K. (2011). Fertilizers (mineral, organic), effect on soil physical properties. *Encyclopedia of Agrophysics (str. 296-299). Springer Netherlands*.

Hernández-Montiel, L. G., Chiquito Contreras, C. J., Murillo Amador, B., Vidal Hernández, L., Quiñones Aguilar, E. E., & Chiquito Contreras, R. G. (2017). Efficiency of two inoculation methods of *Pseudomonas putida* on growth and yield of tomato plants. *Journal of soil science and plant nutrition*, *17*(4), 1003-1012.

Jastrzębska, M., Kostrzewska, M., & Saeid, A. (2022). Conventional agrochemicals: Pros and cons. In *Smart Agrochemicals for Sustainable Agriculture* (pp. 1-28). Academic Press.

Katiyar, D., Hemantaranjan, A., & Singh, B. (2016). Plant growth promoting Rhizobacteria-an efficient tool for agriculture promotion. *Adv Plants Agric Res*, *4*(6), 426-434.

Kumar, A., Patel, J. S., & Meena, V. S. (2018). Rhizospheric microbes for sustainable agriculture: an overview. *Role of rhizospheric microbes in soil*, 1-31.

Lira-Saldivar, R. H., Hernandez, A., Valdez, L. A., Cárdenas, A., Ibarra, L., Hernández, M., & Ruiz, N. (2014). *Azospirillum brasilense* and *Glomus intraradices* co-inoculation stimulates growth and yield of cherry tomato under shadehouse conditions. *Phyton (Buenos Aires)*, *83*(1), 133-138.

Lopes, M. J. D. S., Dias-Filho, M. B., & Gurgel, E. S. C. (2021). Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Frontiers in Sustainable Food Systems*, *5*, 606454.

Mangmang, J. S., Deaker, R., & Rogers, G. (2014). Effects of plant growth promoting rhizobacteria on seed germination characteristics of tomato and lettuce. *Journal of tropical crop science*, *1*(2), 35-40.

Mangmang, J. S., Deaker, R., & Rogers, G. (2015). Optimal plant growth-promoting concentration of *Azospirillum brasilense* inoculated to cucumber, lettuce and tomato seeds varies between bacterial strains. I*srael Journal of Plant Sciences*, 62(3), 145-152.

Meena, K. K., Mesapogu, S., Kumar, M., Yandigeri, M. S., Singh, G., & Saxena, A. K. (2010). Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate-solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea. *Biology and Fertility of Soils*, *46*(2), 169-174.

Msimbira, L. A., & Smith, D. L. (2020). The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. *Frontiers in Sustainable Food Systems*, *4*, 106.

Mwashasha, R. N., Hunja, M., & Kahangi, E. M. (2016, April). Efficiency of plant growth promoting microorganisms on the growth performance of paddy rice. In *Scientific Conference Proceedings* (No. 1).

Paterson, E., Sim, A., Davidson, J., & Daniell, T. J. (2016). Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. *Plant and Soil*, *408*(1), 243-254.

Ratzke, C., & Gore, J. (2018). Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS biology*, *16*(3), e2004248.

Romero-Perdomo, F., Camelo-Rusinque, M., Criollo-Campos, P., & Bonilla-Buitrago, R. (2015). Efecto de la temperatura y el pH en la producción de biomasa de *Azospirillum brasilense* C16 aislada de pasto guinea. *Pastos y Forrajes, 38*(3), 171-175.

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., ... & Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae, 196*, 91-108.

Sargen, M (2020) How microbes grow. *Harvard University*. https://sitn.hms.harvard.edu/flash/2020/how-microbes-grow/

Stark, J. M., & Firestone, M. K. (1995). Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and environmental microbiology*, *61*(1), 218-221.

Tarrand, J. J., Krieg, N. R., & Döbereiner, J. (1978). A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. *Canadian journal of microbiology*, *24*(8), 967-980.

Venturi, V., & Keel, C. (2016). Signaling in the rhizosphere. *Trends in plant science*, *21*(3), 187-198.

Weber, J., Ducousso, M., Tham, F. Y., Nourissier-Mountou, S., Galiana, A., Prin, Y., & Lee, S. K. (2005). Co-inoculation of *Acacia mangium* with *Glomus intraradices* and Bradyrhizobium sp. in aeroponic culture. *Biology and fertility of soils*, *41*(4), 233-239.

Xie, L., Lehvävirta, S., & Valkonen, J. P. (2020). Case study: Planting methods and beneficial substrate microbes effect on the growth of vegetated roof plants in Finland. *Urban Forestry & Urban Greening*, *53*, 126722.

REFERENCES

Abdelaziz, M. E., & Pokluda, R. (2007, September). Response of cucumbers grown on two substrates in an open soilless system to inoculation with microorganisms. In *International Symposium on Growing Media 2007 819* (pp. 157-164).

Abhilash, P. C., Dubey, R. K., Tripathi, V., Gupta, V. K., & Singh, H. B. (2016). Plant growth-promoting microorganisms for environmental sustainability. *Trends in Biotechnology*, *34*(11), 847-850.

Agehara, S., & Warncke, D. D. (2005). Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. *Soil Science Society of America Journal*, *69*(6), 1844-1855.

Aires, A. (2018). Hydroponic production systems: Impact on nutritional status and bioactive compounds of fresh vegetables. *Vegetables: Importance of Quality Vegetables to Human Health*, *55*.

Asghari, B., Khademian, R., & Sedaghati, B. (2020). Plant growth promoting rhizobacteria (PGPR) confer drought resistance and stimulate biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage condition. *Scientia Horticulturae*, *263*, 109132.

Aulakh, M. S., Wassmann, R., Bueno, C., Kreuzwieser, J., & Rennenberg, H. (2001). Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. *Plant biology*, *3*(02), 139-148.

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, cell & environment*, *32*(6), 666-681.

Baldani, J. I., Krieg, N. R., Baldani, V. L. D., Hartmann, A., & Döbereiner, J. (2015). *Azospirillum*. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1-35.

Balestrini, R., Lumini, E., Borriello, R., & Bianciotto, V. (2015). Plant-soil biota interactions. *Soil Microbiol Ecol Biochem*, 311-338.

Balkrishna, A., Sharma, I. P., Arya, V., & Sharma, A. K. (2022). Biologicals and their plant stress tolerance ability. *Symbiosis*, *86*(3), 243-259.

Barassi, C. A., Ayrault, G., Creus, C. M., Sueldo, R. J., & Sobrero, M. T. (2006). Seed inoculation with *Azospirillum* mitigates NaCl effects on lettuce. *Scientia Horticulturae*, *109*(1), 8-14.

Bartelme, R. P., Oyserman, B. O., Blom, J. E., Sepulveda-Villet, O. J., & Newton, R. J. (2018). Stripping away the soil: plant growth promoting microbiology opportunities in aquaponics. *Frontiers in microbiology*, *9*, 8.

Batista, B. D., & Singh, B. K. (2021). Realities and hopes in the application of microbial tools in agriculture. Microbial Biotechnology, 14(4), 1258-1268.

Bashan, Y., & De-Bashan, L. E. (2002). Protection of tomato seedlings against infection by *Pseudomonas syringae* pv. tomato by using the plant growth-promoting bacterium *Azospirillum brasilense*. *Applied and environmental microbiology*, *68*(6), 2637-2643.

Bashan, Y., & De-Bashan, L. E. (2010). How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. *Advances in agronomy*, *108*, 77-136.

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., ... & Zhang, L. (2021). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in plant science*, *10*, 1068.

Begum, N., Wang, L., Ahmad, H., Akhtar, K., Roy, R., Khan, M. I., & Zhao, T. (2021). Co-inoculation of arbuscular mycorrhizal fungi and the plant growth-promoting rhizobacteria improve growth and photosynthesis in tobacco under drought stress by up-regulating antioxidant and mineral nutrition metabolism. *Microbial Ecology*, *83*(4), 971-988.

Bergstrand, K. J. (2022). Organic fertilizers in greenhouse production systems–a review. *Scientia Horticulturae*, *295*, 110855.

Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in microbiology*, *6*, 1559.

Bi, G., Evans, W. B., Spiers, J. M., & Witcher, A. L. (2010). Effects of organic and inorganic fertilizers on marigold growth and flowering. *HortScience*, *45*(9), 1373-1377.

Birhane, E., Sterck, F. J., Fetene, M., Bongers, F., & Kuyper, T. W. (2012). Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. *Oecologia*, *169*(4), 895-904.

Bocatti, C. R., Ferreira, E., Ribeiro, R. A., de Oliveira Chueire, L. M., Delamuta, J. R. M., Kobayashi, R. K. T., ... & Nogueira, M. A. (2022). Microbiological quality analysis of inoculants based on *Bradyrhizobium* spp. and *Azospirillum brasilense* produced “on farm” reveals high contamination with non-target microorganisms. *Brazilian Journal of Microbiology*, *53*(1), 267-280.

Borowik, A., & Wyszkowska, J. (2016). Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant, Soil and Environment*, *62*(6), 250-255.

Bowles, T. M., Barrios-Masias, F. H., Carlisle, E. A., Cavagnaro, T. R., & Jackson, L. E. (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. *Science of the Total Environment*, *566*, 1223-1234.

Britannica, T. 2021. Information Architects of Encyclopedia. Encyclopedia Brit. https://www.britannica.com/facts/auxin

Brunelle, T., Dumas, P., Souty, F., Dorin, B., & Nadaud, F. (2015). Evaluating the impact of rising fertilizer prices on crop yields. *Agricultural economics*, *46*(5), 653-666.

Bücking, H., & Kafle, A. (2015). Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. *Agronomy*, *5*(4), 587-612.

Buzby, J. C., Farah-Wells, H., & Hyman, J. (2014). The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. *USDA-ERS Economic Information Bulletin*, (121).

Chen, J. H. (2006, October). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In *International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use* (Vol. 16, No. 20, pp. 1-11). Land Development Department Bangkok Thailand.

Chojnacka, K., Moustakas, K., & Witek-Krowiak, A. (2020). Bio-based fertilizers: A practical approach towards circular economy. *Bioresource Technology*, *295*, 122223.

Connor, D. J. (2008). Organic agriculture cannot feed the world. *Field Crops Research*, *106*(2), 187.

Costa, A. G., Bertolucci, S. K. V., Chagas, J. H., Ferraz, E. O., & Pinto, J. E. B. P. (2013). Biomass production, yield and chemical composition of peppermint essential oil using different organic fertilizer sources. *Ciência e Agrotecnologia*, *37*, 202-210.

Coughlan, A. P., Dalpé, Y., Lapointe, L., & Piché, Y. (2000). Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Canadian Journal of Forest Research*, *30*(10), 1543-1554.

Curtin, D., Beare, M. H., & Hernandez-Ramirez, G. (2012). Temperature and moisture effects on microbial biomass and soil organic matter mineralization. *Soil Science Society of America Journal*, *76*(6), 2055-2067.

Dasgan, H. Y., & Bozkoylu, A. (2006). Comparison of organic and synthetic-inorganic nutrition of soilless grown summer squash. In *VIII International Symposium on Protected Cultivation in Mild Winter Climates: Advances in Soil and Soilless Cultivation under 747* (pp. 523-528).

de Haas, B. R., Van Gerrewey, T., Perneel, M., Van Labeke, M. C., & Geelen, D. (2021, August). Beneficial microbes for improving circularity and yield in hydroponic crop cultivation. In *II International Symposium on Growing Media, Soilless Cultivation, and Compost Utilization in Horticulture 1317* (pp. 149-156).

Dodd, J. C., Boddington, C. L., Rodriguez, A., Gonzalez-Chavez, C., & Mansur, I. (2000). Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function, and detection. *Plant and soil*, *226*(2), 131-151.

Etesami, H. (2020). Plant–microbe interactions in plants and stress tolerance. In *Plant Life Under Changing Environment* (pp. 355-396). Academic Press.

Fasciglione, G., Casanovas, E. M., Quillehauquy, V., Yommi, A. K., Goñi, M. G., Roura, S. I., & Barassi, C. A. (2015). *Azospirillum* inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. *Scientia Horticulturae, 195,* 154-162.

Fichtner, K., & Schulze, E. D. (1992). The effect of nitrogen nutrition on growth and biomass partitioning of annual plants originating from habitats of different nitrogen availability. *Oecologia*, *92*(2), 236-241.

Franson, R. L., & Bethlenfalvay, G. J. (1989). Infection Unit Method of Vesicular-Arbuscular Mycorrhizal Propagule Determination. *Soil Science Society of America Journal*, *53*(3), 754-756.

Franzluebbers, A. J. (1999). Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. *Applied Soil Ecology*, *11*(1), 91-101.4

Gaskell, M., & Smith, R. (2007). Nitrogen sources for organic vegetable crops. *HortTechnology*, *17*(4), 431-441.

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological research*, *206*, 131-140.

Grobelak, A., Napora, A., & Kacprzak, M. (2015). Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecological Engineering, 84,* 22-28.

Grunert, O., Hernandez-Sanabria, E., Vilchez-Vargas, R., Jauregui, R., Pieper, D. H., Perneel, M., ... & Boon, N. (2016). Mineral and organic growing media have distinct community structure, stability, and functionality in soilless culture systems. *Scientific reports, 6*(1), 1-14.

Gupta, A. R. T. I., & Hussain, N. I. S. R. E. E. N. (2014). A critical study on the use, application and effectiveness of organic and inorganic fertilizers. *Journal of Industrial Pollution Control, 30*(2), 191-194.

Hati, K., & Bandyoopadhay, K. (2011). Fertilizers (mineral, organic), effect on soil physical properties. *Encyclopedia of Agrophysics (str. 296-299). Springer Netherlands*.

Hayat, S., Faraz, A., & Faizan, M. (2017). Root exudates: composition and impact on plant–microbe interaction. *Biofilms in Plant and Soil Health. John Wiley & Sons Ltd*, 179-193.

Hernaández-Esquivel, A. A., Castro-Mercado, E., Valencia-Cantero, E., Alexandre, G., & García-Pineda, E. (2020). Application of *Azospirillum brasilense* lipopolysaccharides to promote early wheat plant growth and analysis of related biochemical responses. *Frontiers in Sustainable Food Systems*, *4*, 579976.

Hernández-Montiel, L. G., Chiquito Contreras, C. J., Murillo Amador, B., Vidal Hernández, L., Quiñones Aguilar, E. E., & Chiquito Contreras, R. G. (2017). Efficiency of two inoculation methods of *Pseudomonas putida* on growth and yield of tomato plants. *Journal of soil science and plant nutrition*, *17*(4), 1003-1012.

Hochmuth, G. J., & Hochmuth, R. C. (2001). Nutrient solution formulation for hydroponic (perlite, rockwool, NFT) tomatoes in Florida. *HS796. Univ. Fla. Coop. Ext. Serv., Gainesville*, 1-10.

Jastrzębska, M., Kostrzewska, M., & Saeid, A. (2022). Conventional agrochemicals: Pros and cons. In *Smart Agrochemicals for Sustainable Agriculture* (pp. 1-28). Academic Press.

Jones, R. D., & Hood, M. A. (1980). Effects of temperature, pH, salinity, and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. *Microbial ecology*, *6*(4), 339-347.

Jurgilevich, A., Birge, T., Kentala-Lehtonen, J., Korhonen-Kurki, K., Pietikäinen, J., Saikku, L., & Schösler, H. (2016). Transition towards circular economy in the food system. *Sustainability*, *8*(1), 69.

Kang, S. M., Shaffique, S., Kim, L. R., Kwon, E. H., Kim, S. H., Lee, Y. H., ... & Lee, I. J. (2021). Effects of organic fertilizer mixed with food waste dry powder on the growth of Chinese cabbage seedlings. *Environments*, *8*(8), 86.

Kano, K., Kitazawa, H., Suzuki, K., Widiastuti, A., Odani, H., Zhou, S., ... & Sato, T. (2021). Effects of organic fertilizer on bok choy growth and quality in hydroponic cultures. *Agronomy*, *11*(3), 491.

Katiyar, D., Hemantaranjan, A., & Singh, B. (2016). Plant growth promoting Rhizobacteria-an efficient tool for agriculture promotion. *Adv Plants Agric Res*, *4*(6), 426-434.

Keswani, C., Singh, S. P., Cueto, L., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., ... & Sansinenea, E. (2020). Auxins of microbial origin and their use in agriculture. *Applied Microbiology and Biotechnology*, *104*(20), 8549-8565.

Khanghahi, M. Y., Ricciuti, P., Allegretta, I., Terzano, R., & Crecchio, C. (2018). Solubilization of insoluble zinc compounds by zinc solubilizing bacteria (ZSB) and optimization of their growth conditions. *Environmental Science and Pollution Research*, *25*(26), 25862-25868.

Khare, E., Chopra, J., & Arora, N. K. (2014). Screening for mcl-PHA-producing fluorescent *Pseudomonads* and comparison of mcl-PHA production under iso-osmotic conditions induced by PEG and NaCl. *Current microbiology*, *68*(4), 457-462.

Kim, J., & Rees, D. C. (1994). Nitrogenase and biological nitrogen fixation. *Biochemistry*, *33*(2), 389-397.

Kim, S. J., Eo, J. K., Lee, E. H., Park, H., & Eom, A. H. (2017). Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. *Mycobiology*, *45*(1), 20-24.

Kozai, T., Niu, G., & Takagaki, M. (Eds.). (2019). *Plant factory: an indoor vertical farming system for efficient quality food production*. Academic press.

Kumar, A., Patel, J. S., & Meena, V. S. (2018). Rhizospheric microbes for sustainable agriculture: an overview. *Role of rhizospheric microbes in soil*, 1-31.

Kurt, D., & Ayan, A. K. (2014). Effect of the different organic fertilizer sources and doses on yield in organic tobacco (*Nicotiana tabacum* L.) production.

Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytologist*, *198*(3), 656-669.

Li, S. X., Wang, Z. H., & Stewart, B. A. (2013). Responses of crop plants to ammonium and nitrate N. *Advances in agronomy, 118*, 205-397.

Li, T., Lin, G., Zhang, X., Chen, Y., Zhang, S., & Chen, B. (2014). Relative importance of an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) and root hairs in plant drought tolerance. *Mycorrhiza*, *24*(8), 595-602.

Linn, D. M., & Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, *48*(6), 1267-1272.

Lira-Saldivar, R. H., Hernandez, A., Valdez, L. A., Cárdenas, A., Ibarra, L., Hernández, M., & Ruiz, N. (2014). *Azospirillum brasilense* and *Glomus intraradices* co-inoculation stimulates growth and yield of cherry tomato under shadehouse conditions. *Phyton (Buenos Aires), 83*(1), 133-138.

Lopes, M. J. D. S., Dias-Filho, M. B., & Gurgel, E. S. C. (2021). Successful plant growth-promoting microbes: inoculation methods and abiotic factors. *Frontiers in Sustainable Food Systems*, *5*, 606454.

Lopez-Bucio, J., De la Vega, O. M., Guevara-Garcia, A., & Herrera-Estrella, L. (2000). Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. *Nature biotechnology*, *18*(4), 450-453.

Lucas García, J. A., Barbas, C., Probanza, A., Barrientos, M. L., & Gutierrez Mañero, F. J. (2001). Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *12*(5), 305-311.

Macintyre, O. J., Trevors, J. T., Dixon, M. A., & Cottenie, K. (2011). Application of plant growth promoting rhizobacteria in a hydroponics system for advanced life support in space. *Acta horticulturae,* (893), 1285-1292.

Majeed, A., Abbasi, M. K., Hameed, S., Yasmin, S., Hanif, M. K., Naqqash, T., & Imran, A. (2018). *Pseudomonas* sp. AF-54 containing multiple plant beneficial traits acts as growth enhancer of *Helianthus annuus* L. under reduced fertilizer input. *Microbiological research*, *216*, 56-69.

Majid, M., Khan, J. N., Shah, Q. M. A., Masoodi, K. Z., Afroza, B., & Parvaze, S. (2021). Evaluation of hydroponic systems for the cultivation of Lettuce (*Lactuca* *sativa* L., var. Longifolia) and comparison with protected soil-based cultivation. *Agricultural Water Management*, *245*, 106572.

Mangmang, J. S., Deaker, R., & Rogers, G. (2014). Effects of plant growth promoting rhizobacteria on seed germination characteristics of tomato and lettuce. *Journal of tropical crop science*, *1*(2), 35-40.

Mangmang, J. S., Deaker, R., & Rogers, G. (2015). Early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated by soaking and drenching. *Horticultural Science*, *42*(1), 37-46.

Meena, K. K., Mesapogu, S., Kumar, M., Yandigeri, M. S., Singh, G., & Saxena, A. K. (2010). Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate-solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea. *Biology and Fertility of Soils*, *46*(2), 169-174.

Mickan, B. S., Ren, A. T., Buhlmann, C. H., Ghadouani, A., Solaiman, Z. M., Jenkins, S., ... & Ryan, M. H. (2022). Closing the circle for urban food waste anaerobic digestion: The use of digestate and biochar on plant growth in potting soil. *Journal of Cleaner Production*, *347*, 131071.

Mikiciuk, G., Sas-Paszt, L., Mikiciuk, M., Derkowska, E., Trzciński, P., Głuszek, S., ... & Rudnicka, J. (2019). Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and rhizosphere bacteria. *Mycorrhiza*, *29*(5), 489-501.

Mishra, N., & Sundari, S. K. (2013). Native PGPMs as bioinoculants to promote plant growth: response to PGPM inoculation in principal grain and pulse crops. *International Journal of Agriculture Food Science & Technology*, *4*(10), 1055-1064.

Mohammad, A., Mitra, B., & Khan, A. G. (2004). Effects of sheared-root inoculum of *Glomus intraradices* on wheat grown at different phosphorus levels in the field. *Agriculture, ecosystems & environment*, *103*(1), 245-249.

Moncada, A., Vetrano, F., & Miceli, A. (2020). Alleviation of salt stress by plant growth-promoting bacteria in hydroponic leaf lettuce. *Agronomy*, *10*(10), 1523.

Moncada, A., Miceli, A., & Vetrano, F. (2021). Use of plant growth-promoting rhizobacteria (PGPR) and organic fertilization for soilless cultivation of basil. *Scientia Horticulturae*, *275*, 109733.

Msimbira, L. A., & Smith, D. L. (2020). The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. *Frontiers in Sustainable Food Systems*, *4*, 106.

Mwashasha, R. N., Hunja, M., & Kahangi, E. M. (2016, April). Efficiency of plant growth promoting microorganisms on the growth performance of paddy rice. In *Scientific Conference Proceedings* (No. 1).

Neumann, G., Bott, S., Ohler, M. A., Mock, H. P., Lippmann, R., Grosch, R., & Smalla, K. (2014). Root exudation and root development of lettuce (Lactuca sativa L. cv. Tizian) as affected by different soils. *Frontiers in microbiology*, *5*, 2.

Nosheen, S., Ajmal, I., & Song, Y. (2021). Microbes as biofertilizers, a potential approach for sustainable crop production. *Sustainability*, *13*(4), 1868.

Oelkers, E. H., & Valsami-Jones, E. (2008). Phosphate mineral reactivity and global sustainability. *Elements*, *4*(2), 83-87.

Oljira, A. M., Hussain, T., Waghmode, T. R., Zhao, H., Sun, H., Liu, X., ... & Liu, B. (2020). *Trichoderma* enhances net photosynthesis, water use efficiency, and growth of wheat (*Triticum aestivum* L.) under salt stress. *Microorganisms*, *8*(10), 1565.

Ortíz-Castro, R., Contreras-Cornejo, H. A., Macías-Rodríguez, L., & López-Bucio, J. (2009). The role of microbial signals in plant growth and development. *Plant signaling & behavior*, *4*(8), 701-712.

Paradiso, R., Arena, C., De Micco, V., Giordano, M., Aronne, G., & De Pascale, S. (2017). Changes in leaf anatomical traits enhanced photosynthetic activity of soybean grown in hydroponics with plant growth-promoting microorganisms. *Frontiers in Plant Science*, *8*, 674.

Paterson, E., Sim, A., Davidson, J., & Daniell, T. J. (2016). Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. *Plant and Soil*, *408*(1), 243-254.

Paull, J. (2009). A century of synthetic fertilizer: 1909-2009.

Pelayo Lind, O., Hultberg, M., Bergstrand, K. J., Larsson-Jönsson, H., Caspersen, S., & Asp, H. (2021). Biogas digestate in vegetable hydroponic production: pH dynamics and pH management by controlled nitrification. *Waste and Biomass Valorization*, *12*(1), 123-133.

Phibunwatthanawong, T., & Riddech, N. (2019). Liquid organic fertilizer production for growing vegetables under hydroponic condition. *International Journal of Recycling of Organic Waste in Agriculture*, *8*(4), 369-380.

Ratzke, C., & Gore, J. (2018). Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS biology*, *16*(3), e2004248.

Romero-Perdomo, F., Camelo-Rusinque, M., Criollo-Campos, P., & Bonilla-Buitrago, R. (2015). Efecto de la temperatura y el pH en la producción de biomasa de *Azospirillum brasilense* C16 aislada de pasto guinea. *Pastos y Forrajes*, *38*(3), 171-175.

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., ... & Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae*, *196*, 91-108.

Rudrappa, T., Czymmek, K. J., Paré, P. W., & Bais, H. P. (2008). Root-secreted malic acid recruits beneficial soil bacteria. *Plant physiology*, *148*(3), 1547-1556.

Saeed, K. S., Ahmed, S. A., Hassan, I. A., & Ahmed, P. H. (2015). Effect of bio-fertilizer and chemical fertilizer on growth and yield in cucumber (*Cucumis sativus*) in green house condition. *Pak J Biol Sci*, *18*(3), 129-134.

Saijai, S., Ando, A., Inukai, R., Shinohara, M., & Ogawa, J. (2016). Analysis of microbial community and nitrogen transition with enriched nitrifying soil microbes for organic hydroponics. *Bioscience, biotechnology, and biochemistry*, *80*(11), 2247-2254.

Sargen, M (2020) How microbes grow. *Harvard University*. https://sitn.hms.harvard.edu/flash/2020/how-microbes-grow/

Saubidet, M. I., Fatta, N., & Barneix, A. J. (2002). The effect of inoculation with *Azospirillum brasilense* on growth and nitrogen utilization by wheat plants. *Plant and soil*, *245*(2), 215-222.

Savci, S. (2012). Investigation of effect of chemical fertilizers on environment. *Apcbee Procedia*, *1*, 287-292.

Sharma, N., Acharya, S., Kumar, K., Singh, N., & Chaurasia, O. P. (2018). Hydroponics as an advanced technique for vegetable production: An overview. *Journal of Soil and Water Conservation*, *17*(4), 364-371.

Shaul-Keinan, O., Gadkar, V., Ginzberg, I., Grünzweig, J. M., Chet, I., Elad, Y., ... & Kapulnik, Y. (2002). Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with Glomus intraradices. *New Phytologist*, *154*(2), 501-507.

Sheridan, C., Depuydt, P., De Ro, M., Petit, C., Van Gysegem, E., Delaere, P., ... & Geelen, D. (2017). Microbial community dynamics and response to plant growth-promoting microorganisms in the rhizosphere of four common food crops cultivated in hydroponics. *Microbial ecology*, *73*(2), 378-393.

Shinohara, M., Aoyama, C., Fujiwara, K., Watanabe, A., Ohmori, H., Uehara, Y., & Takano, M. (2011). Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. *Soil science and plant nutrition*, *57*(2), 190-203.

Silgram, M., & Shepherd, M. A. (1999). The effects of cultivation on soil nitrogen mineralization. *Advances in agronomy*, *65*, 267-311.

Smith, S. R., & Hadley, P. (1989). A comparison of organic and inorganic nitrogen fertilizers: Their nitrate-N and ammonium-N release characteristics and effects on the growth response of lettuce (*Lactuca sativa* L. cv. Fortune). *Plant and soil*, *115*(1), 135-144.

Soretire, A. A., Adeyemi, N. O., Atayese, M. O., Sakariyawo, O. S., & Adewunmi, A. (2020). Nodulation and biological nitrogen fixation in soybean (*Glycine max* L.) as influenced by phosphorus fertilization and arbuscular mycorrhizal inoculation. *Acta Universitatis Sapientiae, Agriculture and Environment*, *12*(1), 22-44.

Souza, R. D., Ambrosini, A., & Passaglia, L. M. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and molecular biology*, *38*, 401-419.

Stark, J. M., & Firestone, M. K. (1995). Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and environmental microbiology*, *61*(1), 218-221.

Steenhoudt, O., & Vanderleyden, J. (2000). *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS microbiology reviews*, *24*(4), 487-506.

Stoknes, K., Wojciechowska, E., Jasińska, A., Gulliksen, A., & Tesfamichael, A. A. (2017). Growing vegetables in the circular economy; cultivation of tomatoes on green waste compost and food waste digestate. In *International Symposium on Greener Cities for More Efficient Ecosystem Services in a Climate Changing World 1215* (pp. 389-396).

Stoknes, K., Scholwin, F., Jasinska, A., Wojciechowska, E., Mleczek, M., Hanc, A., & Niedzielski, P. (2019). Cadmium mobility in a circular food-to-waste-to-food system and the use of a cultivated mushroom (Agaricus subrufescens) as a remediation agent. *Journal of environmental management*, *245*, 48-54.

Tarrand, J. J., Krieg, N. R., & Döbereiner, J. (1978). A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. *Canadian journal of microbiology*, *24*(8), 967-980.

Tesfaye, M., Dufault, N. S., Dornbusch, M. R., Allan, D. L., Vance, C. P., & Samac, D. A. (2003). Influence of enhanced malate dehydrogenase expression by alfalfa on diversity of rhizobacteria and soil nutrient availability. *Soil Biology and Biochemistry*, *35*(8), 1103-1113.

Tien, T. M., Gaskins, M. H., & Hubbell, D. (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and environmental microbiology*, *37*(5), 1016-1024.

Vande Broek, A., Keijers, V., & Vanderleyden, J. (1996). Effect of oxygen on the free-living nitrogen fixation activity and expression of the *Azospirillum brasilense* NifH gene in various plant-associated diazotrophs. *Symbiosis*.

Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., & Maggio, A. (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*, *4*(1), 1-12.

Venturi, V., & Keel, C. (2016). Signaling in the rhizosphere. *Trends in plant science*, *21*(3), 187-198.

Wada, T. (2019). Theory and technology to control the nutrient solution of hydroponics. In *Plant Factory Using Artificial Light* (pp. 5-14). Elsevier.

Watts-Williams, S. J., Turney, T. W., Patti, A. F., & Cavagnaro, T. R. (2014). Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas. *Plant and Soil*, *376*(1), 165-175.

Weber, J., Ducousso, M., Tham, F. Y., Nourissier-Mountou, S., Galiana, A., Prin, Y., & Lee, S. K. (2005). Co-inoculation of *Acacia mangium* with *Glomus intraradices* and *Bradyrhizobium* sp. in aeroponic culture. *Biology and fertility of soils*, *41*(4), 233-239.

Wu, Q. S., Liu, C. Y., Zhang, D. J., Zou, Y. N., He, X. H., & Wu, Q. H. (2016). Mycorrhiza alters the profile of root hairs in trifoliate orange. *Mycorrhiza*, *26*(3), 237-247.

Xie, L., Lehvävirta, S., & Valkonen, J. P. (2020). Case study: Planting methods and beneficial substrate microbes effect on the growth of vegetated roof plants in Finland. *Urban Forestry & Urban Greening*, *53*, 126722.

Xie, M. M., Chen, S. M., Zou, Y. N., Srivastava, A. K., Rahman, M. M., Wu, Q. S., & Kuča, K. (2021). Effects of *Rhizophagus intraradices* and *Rhizobium trifolii* on growth and N assimilation of white clover. *Plant Growth Regulation*, *93*(3), 311-318.

Zahradník, A., & Petrikova, K. (2007). Effect of alternative organic fertilizers on the nutritional value and yield of head cabbage. *Hort. Sci.(Prague)*, *34*(2), 65-71.

Zaman, S., Pramanick, P., & Mitra, A. (2019). Chemical fertilizer. *Department of Marine Science, University of Calcutta: Kolkata, India*