Combining solid digestate with microorganisms and a biostimulant for a potentially enhanced quality of soilless organically grown tomato plants

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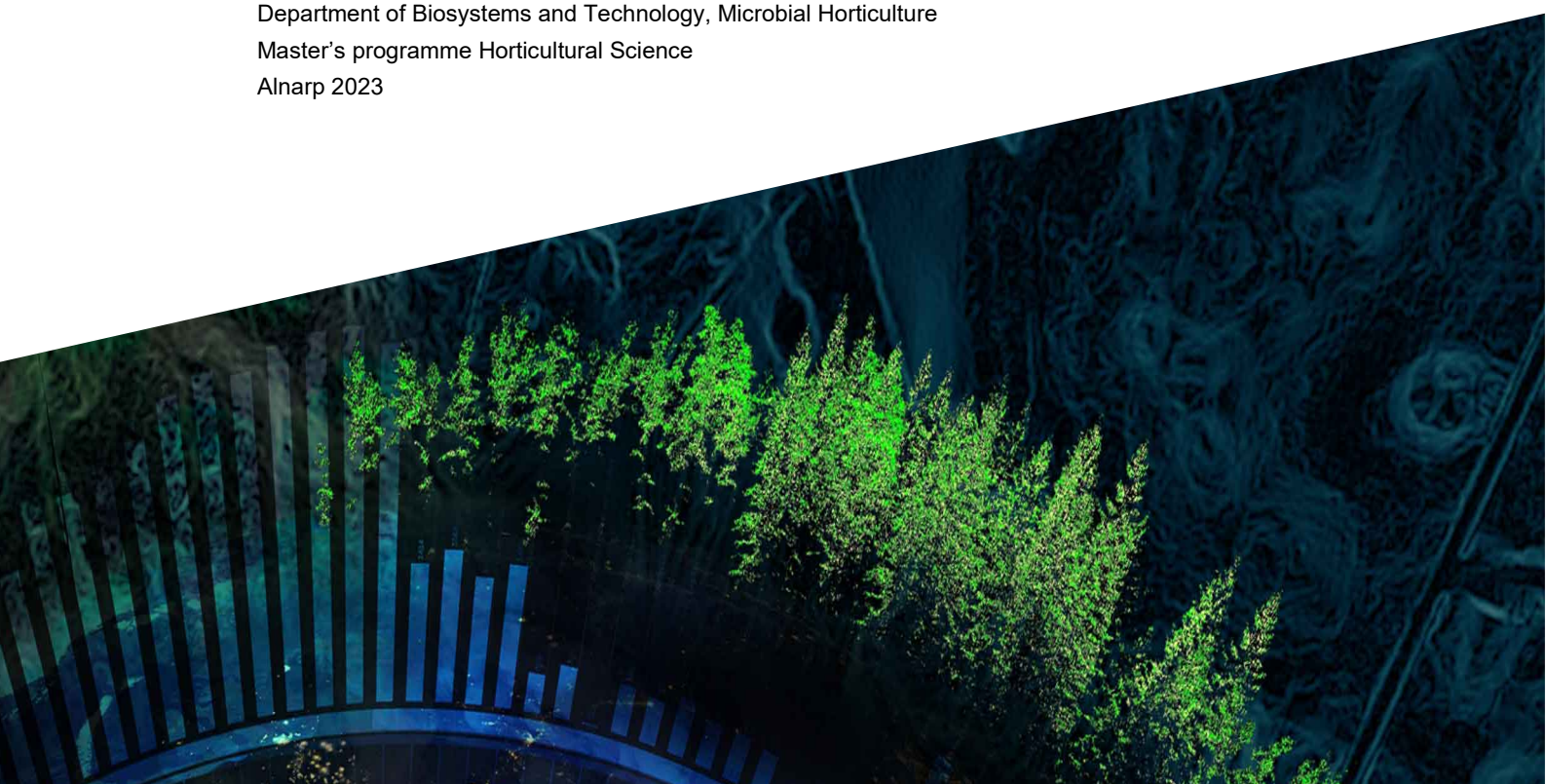

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Abstract

Sustainable agricultural practices are at the forefront of addressing global food security challenges while minimizing environmental impact. This research aimed to contribute to these efforts, focusing on tomato (*Solanum lycopersicum* L.) cultivation, a globally significant crop known for its nutritional benefits and economic value. The main objectives of this study were to explore the use of solid digestate as an alternative to peat, which is non-renewable and environmentally detrimental, and to assess the effect of *Trichoderma afroharzianum* T-22, *Bacillus amyloliquefaciens*, and poly-β-hydroxybutyrate (PHB) on plant growth, health, and productivity. The utilization of solid digestate showed promising potential for reducing dependence on peat. Bio-inoculation with *T. afroharzianum* T-22 and *B. amyloliquefaciens* and the application of PHB were found to influence parameters like chlorophyll content and nutrient uptake, even though they did not show significant differences regarding plant biomass. Intriguing interactions between different treatments and their effect on microbial colony forming unit (CFU) counts in the substrate were unveiled, highlighting the complex interplay between microbial communities and plant health. These findings underscore the potential of integrating renewable substrates and beneficial microorganisms in tomato cultivation towards more sustainable and efficient agricultural practices. They also illuminate a path for future research, particularly in the realm of organic production, where such strategies can contribute significantly to optimizing plant productivity while preserving environmental integrity. The exploration of more combinations of treatments, a wider range of plant species, as well as the elimination of harmful residues is recommended to broaden the applicability of the findings.

Keywords: Solid digestate, *Trichoderma afroharzianum* T-22*, Bacillus amyloliquefaciens*, poly-β-hydroxybutyrate (PHB)

Table of contents

List of tables

List of figures

Abbreviations

CCI	Chlorophyll content index
NH4-N	Ammonium to Nitrogen relation
NO3-N	Nitrate to Nitrogen relation
PHB	Poly-β-hydroxybutyrate
PO4-P	Phosphate to Phosphorus relation
SLU	Swedish University of Agricultural Sciences
TB	*T. afroharzianum* T-22 + *B. amyloliquefaciens*
TBA	*T. afroharzianum* T-22 + *B. amyloliquefaciens* + Albit

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a globally cultivated crop with significant economic and nutritional value, serving as an essential source of vitamins, minerals, and antioxidants in human diets (FAOSTAT, 2021; Raiola et al., 2014). The worldwide demand for tomatoes has led to an expansion of cultivation areas, encompassing a range of environments and production systems. However, tomato production faces numerous challenges, including various biotic and abiotic stress factors, such as pathogens, pests, drought, salinity, and nutrient deficiencies, which can impact plant growth, yield, and quality (Ronga et al., 2017).

In this context, sustainable approaches to improve tomato plant growth, resilience, and productivity have become a priority in agricultural research, aiming to reduce the dependence on chemical inputs, minimize the environmental impact, and enhance overall crop performance (Yakhin et al., 2017; Colla et al., 2015). Among these approaches is the application of by-products from anaerobic digestion, such as solid digestate, as a fertilizer and substrate. Solid digestate is a nutrient-rich material, containing organic matter, nitrogen, phosphorus, and other essential elements, which can improve soil fertility, enhance nutrient availability, and stimulate plant growth (Möller & Müller, 2012; Nkoa, 2014). Moreover, the utilization of solid digestate in agriculture contributes to the circular economy by recycling waste materials and reducing the reliance on synthetic fertilizers (Alburquerque et al., 2012).

Another promising approach involves the use of plant growth-promoting microorganisms (PGPMs), such as *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*, which have been shown to promote plant growth, induce systemic resistance, and enhance tolerance to various stress factors through a range of direct and indirect mechanisms (Harman et al., 2004; Chowdhury et al., 2015a). Additionally, the use of natural biopolymers, such as poly-β-hydroxybutyrate (PHB), has gained attention in recent years for its potential in enhancing plant growth and stress tolerance. PHB is a biodegradable polymer produced by certain microorganisms, which can accumulate in plant tissues and modulate various

physiological processes, including photosynthesis, respiration, and antioxidant defense mechanisms (Kour et al., 2020).

In this thesis, the interactions between tomato plants that are grown in solid digestate, and these various growth-promoting agents will be explored, examining their potential benefits, enhancements, and underlying mechanisms. By investigating the effects of PGPMs, solid digestate, and PHB on tomato plant growth, yield, and stress tolerance, I aim to provide insights into the development of integrated management strategies that can contribute to the sustainability and resilience of tomato production systems worldwide.

1.1 Background

1.1.1 Solid Digestate as a fertilizer and substrate

Solid digestate, derived from the anaerobic digestion process, has garnered interest as an eco-friendly and sustainable option for use as a fertilizer and substrate in agriculture (Möller & Müller, 2012). Anaerobic digestion involves the breakdown of organic matter by microorganisms in an oxygen-deprived environment, leading to the production of biogas and digestate (Nkoa, 2014). This process is commonly utilized for waste management, particularly in the treatment of agricultural, municipal, and industrial wastes.

The solid digestate is a heterogeneous mixture composed of undigested organic matter, nutrients (including nitrogen, phosphorus, and potassium), and microbial biomass (Odlare et al., 2011; Möller & Müller, 2012). It is obtained after the separation of liquid and solid fractions of the digestate, and its composition varies depending on the feedstock and anaerobic digestion conditions (Alburquerque et al., 2012; Nkoa, 2014). Notably, solid digestate has a high organic matter content, which can enhance soil structure, water retention, and cation exchange capacity when used as a soil amendment (Odlare et al., 2011; Möller & Müller, 2012).

Moreover, solid digestate contains essential plant nutrients and trace elements, such as nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients (Alburquerque et al., 2012). These nutrients are often present in forms that are readily available to plants, thereby improving plant nutrition and growth (Tambone et al., 2010; Ronga et al., 2017). In addition, the microbial biomass in solid digestate can contribute to enhanced nutrient cycling and soil biological activity, promoting a healthy soil ecosystem (Odlare et al., 2011).

The use of solid digestate as a soil amendment has demonstrated numerous benefits, including improved soil fertility, increased nutrient availability, and enhanced plant growth (Tambone et al., 2010; Ronga et al., 2017). Tallou et al. (2022) observed that applying anaerobic digestate to soil positively impacted tomato growth, fruit quality, and soil microbial biomass. Furthermore, the application of solid digestate can mitigate some environmental risks associated with conventional fertilizers, such as nutrient leaching, groundwater contamination, and greenhouse gas emissions (Nkoa, 2014).

Research has been conducted to understand the appropriate proportion of solid digestate to be used in various plant cultivation systems. For instance, Hultberg et al (2022) found that the use of solid digestate at a rate of 30% of the total substrate was beneficial for a combined cultivation of mushrooms and basil growth. In another study on blueberry (*Vaccinium corymbosum* L.) cultivation, Bignami et al. (2022) reported that a 20-40% digestate to substrate ratio was optimal for root development, had lower degree of defoliaton, and had the best nitrogen balance index. Similarly, Greco et al (2021) found that the inclusion of solid digestate at 40% of the total substrate did not negatively affect sage (*Salvia officinalis* L.) cultivation and had similar results with common peat substrate. He also mentioned that due to the higher values of the electrical conductivity of the substrates obtained from anaerobic digestion processes, such substrates must be used with caution. These studies demonstrate that the proportion of solid digestate can vary depending on the plant species and the specific cultivation system, but generally ranges from 20% to 40% of the total substrate.

Challenges of Solid Digestate and the role of Pyralids

Despite the numerous advantages, there are potential challenges associated with the use of solid digestate in agriculture, such as the presence of contaminants (e.g., heavy metals, pathogens, and organic pollutants), odors, and variability in nutrient content (Möller & Müller, 2012; Nkoa, 2014). Those challenges exist due to the lack of proper management practices, including the kind of feedstock that is selected, pre-treatment, and post-treatment, which results in potentially unsafe and inefficient use of solid digestate in agricultural systems (Möller & Müller, 2012). Moreover, the use of solid digestate as a sole substrate for plant growth presents a series of challenges primarily related to its high pH levels. The process of anaerobic digestion often results in a digestate with high pH, usually above 8.0, which can result in the precipitation and subsequent unavailability of certain nutrients, such as phosphorus and certain trace elements, which are vital for plant growth (Möller & Müller, 2012). This high pH can also limit the activity of many soil microorganisms, disrupting the nutrient cycling processes in the soil ecosystem (Insam et al., 2015). Furthermore, high pH can cause nutrient imbalances, leading

to nutrient deficiencies or toxicities, which can negatively impact plant growth and development (Möller & Stinner, 2009). Therefore, while solid digestate presents an attractive sustainable alternative to conventional fertilizers due to its high nutrient content and organic matter, its high pH presents significant challenges to its direct use as a substrate in agriculture.

A significant challenge of solid digestate is the presence of pyralids. They are a group of herbicides commonly used to control broadleaf weeds in various agricultural settings (Heap, 2021). Clopyralid, a member of this group, has been reported to persist in compost and other organic substrates, raising concerns about its unintended effects on non-target plants, including tomatoes (Brinton, 2000). In Sweden, since 2020, hobby growers have experienced adverse effects on their plants due to the presence of clopyralid residues in the organic substrates, including solid digestate, they used (Swedish Chemicals Agency, 2020). Although the detected levels of clopyralid were below the maximum allowed concentration for a substrate to be considered organic, it was still observed to negatively impact tomato plants (Swedish Chemicals Agency, 2020). Clopyralid has been shown to affect various aspects of tomato plant growth and development. When present in the growing medium, it can be taken up by the roots and transported throughout the plant, causing damage to the roots, stems, and leaves (Bromilow et al., 1990). Symptoms of clopyralid exposure in tomato plants may include stunted growth, leaf curling, chlorosis, necrosis, and reduced fruit yield (Chang et al, 2017). Furthermore, the herbicide can interfere with the normal physiological processes of tomato plants, including photosynthesis, respiration, and nutrient uptake (Wauchope et al., 2005). The presence of clopyralid in solid digestate may also affect the interactions between tomato plants and plant growth-promoting agents such as *Trichoderma afroharzianum* T-22, *Bacillus amyloliquefaciens*, and poly-β-hydroxybutyrate (PHB). Although direct interactions between these agents and clopyralid have not been widely studied, it is possible that the herbicide could interfere with their beneficial effects on plant growth and stress tolerance.

Summarizing

Overall, solid digestate has promising potential as a sustainable and eco-friendly alternative to conventional fertilizers and substrates in agriculture. Its origins in the anaerobic digestion process make it a valuable by-product, repurposing waste materials and providing an effective method of waste management. The benefits of solid digestate, such as enhanced soil fertility, increased nutrient availability, and improved plant growth, make it a viable option for sustainable agriculture (Tambone et al., 2010).

However, it is crucial to adopt proper management practices to ensure the safe and efficient use of solid digestate. By considering factors such as feedstock selection, pre-treatment, and post-treatment, the challenges associated with solid digestate application, such as potential contamination and variability in nutrient content, can be addressed (Möller & Müller, 2012; Nkoa, 2014). As research into the properties and applications of solid digestate continues, its role in promoting sustainable agricultural practices will likely expand, contributing to a more environmentally responsible approach to crop production.

1.1.2 Peat and its environmental impact

Peat is a natural organic material that accumulates in waterlogged conditions, primarily in peatlands or mires, through the slow decomposition of plant matter under anaerobic conditions (Alexander et al., 2008). Peat has long been used as a soil amendment and growing media component in agriculture and horticulture due to its excellent water retention, aeration, and buffering properties (Alexander et al., 2008). However, the extraction and use of peat for horticultural purposes have raised significant environmental concerns. Peat extraction contributes to the degradation of peatlands, which are vital ecosystems for carbon sequestration, water regulation, and biodiversity conservation (Paoli et al., 2022). Moreover, peat extraction and drainage lead to the release of stored carbon into the atmosphere, exacerbating climate change (Paoli et al., 2022). Consequently, efforts to reduce peat consumption in agriculture and horticulture have become crucial to promote more sustainable practices.

1.1.3 Pumice as a growing media component

Pumice is a lightweight, porous volcanic rock formed during explosive volcanic eruptions (Pérez-Urrestarazu et al., 2019). Due to its unique physical properties, such as high porosity, low bulk density, and excellent drainage capacity, pumice has gained interest as an alternative to peat in horticulture (Pérez-Urrestarazu et al., 2019). Pumice can improve soil aeration, water retention, and nutrient availability, positively influencing plant growth and development (Pérez-Urrestarazu et al., 2019). Studies have reported beneficial effects of pumice incorporation in substrates for tomato cultivation. For instance, Mitsanis et al. (2021) found that the use of pumice as a substrate component improved tomato growth, yield, and fruit quality, suggesting its potential as a sustainable alternative to peat.

1.1.4 *Trichoderma afroharzianum* T-22 as a plant growth promoter

Trichoderma afroharzianum T-22, a beneficial filamentous fungus belonging to the genus Trichoderma, has garnered significant attention due to its ability to promote plant growth and protect plants against a wide range of pathogens (Vinale et al., 2008). *Trichoderma spp*., including *T. afroharzianum* T-22, are ubiquitous in nature, primarily inhabiting the rhizosphere and soil, where they establish symbiotic relationships with plants and play vital roles in plant health and development (Harman et al., 2004; Druzhinina et al., 2011). These fungi are also commercially important and have been developed as biocontrol agents and biofertilizers in sustainable agriculture (Chen et al., 2011).

The mechanisms of action of *Trichoderma spp*. are diverse and multifaceted, encompassing direct and indirect strategies that contribute to their plant growth-promoting and biocontrol properties. Direct mechanisms include mycoparasitism, where *Trichoderma spp*. attack and parasitize other fungi, and competition for nutrients and space, which can limit the proliferation of pathogens (Harman et al., 2004; Vinale et al., 2008). *Trichoderma spp*. also produce a wide array of antimicrobial compounds, such as cell wall-degrading enzymes and secondary metabolites, which can inhibit the growth and virulence of plant pathogens (Vinale et al., 2008; Zeilinger et al., 2016).

In addition to their biocontrol properties, *Trichoderma spp*. have been reported to enhance plant growth directly by producing phytohormones, such as auxins, cytokinins, and gibberellins, which can modulate plant development and stress responses (Contreras-Cornejo et al., 2009; Viterbo et al., 2010). For example, *T. afroharzianum* T-22 was shown to produce indole-3-acetic acid (IAA), an auxin involved in cell elongation, root development, and stress tolerance (Joo et al., 2005; Kottb et al., 2015). Indirect mechanisms of plant growth promotion by *Trichoderma spp*. include improving nutrient availability and uptake, by solubilizing minerals such as phosphorus and iron, and enhancing nitrogen fixation through interactions with plant-associated bacteria (Altomare et al., 1999; Viterbo et al., 2010).

Numerous studies have demonstrated the positive effects of *T. afroharzianum* on plant growth and yield across various crops. For instance, Contreras-Cornejo et al. (2009) reported that *T. virens* promoted lateral root growth in *Arabidopsis* through an auxin-dependent mechanism. Similarly, Doni et al. (2014) demonstrated that *Trichoderma spp* inoculation increased biomass production, root length, and nutrient uptake in rice plants, indicating its potential as a plant growth-promoting agent. These findings underscore the value of *T. afroharzianum* T-22 in promoting

plant growth and health, and its potential application in sustainable agriculture and crop improvement.

1.1.5 *Bacillus amyloliquefaciens* as a plant growth promoter and phosphorus solubilizer

Bacillus amyloliquefaciens, a gram-positive, spore-forming bacterium, is widely found in soil, plant rhizospheres, and plant tissues (Chowdhury et al., 2015a). It belongs to the *Bacillus subtilis* group, which comprises plant-associated bacteria with plant growth-promoting and biocontrol properties (Borriss, 2011). Its beneficial effects on plant growth and health can be attributed to its multiple modes of action, including direct stimulation of plant growth, nutrient solubilization, and biocontrol against pathogens (Chowdhury et al., 2015a; Pérez-García et al., 2011).

B. amyloliquefaciens produces phytohormones, such as indole-3-acetic acid (IAA), which can stimulate plant growth by modulating cell division and elongation (Idris et al., 2007; Spaepen et al., 2007). Additionally, this bacterium can facilitate plant nutrient uptake by solubilizing inorganic phosphorus, which is often present in unavailable forms in soils (Rodriguez et al., 2006). *B. amyloliquefaciens* achieves this through the production of organic acids, such as gluconic acid and 2-ketogluconic acid, which can chelate and solubilize insoluble phosphates (Khan et al., 2010; Sharma et al., 2013).

The biocontrol properties of *B. amyloliquefaciens* are attributed to its production of various antimicrobial compounds, such as lipopeptides, polyketides, and bacteriocins, which can inhibit the growth of plant pathogens (Chowdhury et al., 2015a; Pérez-García et al., 2011). In addition to direct antagonism, *B. amyloliquefaciens* can induce systemic resistance in host plants, thereby enhancing their ability to defend against pathogens (Choudhary et al., 2007). For example, *B. amyloliquefaciens* SQR9 was reported to increase cucumber growth and resistance to *Fusarium* wilt disease, possibly through the induction of systemic resistance and modulation of the plant immune system (Shao et al., 2015).

B. amyloliquefaciens has demonstrated its plant growth-promoting and biocontrol properties in various crops, including cereals, legumes, and vegetables (Chowdhury et al., 2015a; Khan et al., 2010). Its multifunctional characteristics make it a promising candidate for sustainable agriculture, offering an environmentally friendly alternative to chemical fertilizers and pesticides (Borriss, 2011; Chowdhury et al., 2015a).

1.1.6 Poly-β-hydroxybutyrate (PHB) and its effects on plants

Origin and Production of PHB

Poly-β-hydroxybutyrate (PHB) is a natural biopolymer produced by a wide range of microorganisms, such as bacteria and algae, as a carbon and energy storage compound under nutrient-limiting conditions (Sudesh et al., 2000; Kourmentza et al., 2017). The biosynthesis of PHB is mediated by the enzyme PHB synthase, which polymerizes the precursor 3-hydroxybutyryl-CoA into PHB (Steinbüchel & Hein, 2001). Some notable PHB-producing bacteria include members of the genera *Ralstonia*, *Bacillus*, and *Azotobacter* (Kourmentza et al., 2017). In recent years, the production of PHB has gained significant interest due to its potential applications as a biodegradable alternative to petroleum-based plastics, addressing environmental concerns associated with plastic pollution (Sudesh et al., 2000; Kourmentza et al., 2017). In this experiment, PHB is applied to the plants through a commercial organic biostimulant, called Albit, which is manufactured by Albit Scientific and Industrial LLC.

PHB and Plant Growth Promotion

The potential of PHB to promote plant growth and stress tolerance has been investigated in various studies. The exact mechanisms through which PHB affects plant growth are not yet fully understood, but several hypotheses have been proposed. One possibility is that PHB may act as a signalling molecule, regulating plant growth and stress responses. Additionally, PHB might enhance nutrient availability and uptake by plants by stimulating the activity of plant growth-promoting microorganisms in the rhizosphere (Zhang et al., 2016a). Another potential mechanism is the interaction of PHB with plant cell membranes, altering their permeability and functionality (Poirier et al., 1992).

PHB in Plant Stress Tolerance

Several studies have reported the positive effects of PHB on plant stress tolerance. Selim et al. (2021) investigated the effects of poly-β-hydroxybutyrate (PHB) produced by *Rhizobium phaseoli* on the abiotic stress-induced resistance in common bean plants. The study aimed to determine the role of PHB in enhancing the tolerance of bean plants to salinity and drought stress conditions. The results demonstrated that the application of PHB-producing *R. phaseoli* significantly improved the growth and physiological parameters of the bean plants under both salinity and drought stress conditions. The plants exhibited increased antioxidant enzyme activities, proline accumulation, and reduced lipid peroxidation, indicating enhanced stress tolerance (Selim et al., 2021). Furthermore, the application of PHB

led to an increase in nitrogen fixation and the uptake of essential nutrients, such as phosphorus, potassium, and calcium, in the common bean plants. These findings suggest that the use of PHB-producing *R. phaseoli* can effectively promote abiotic stress-induced resistance in common bean plants, making it a promising strategy for improving crop productivity under challenging environmental conditions (Selim et al., 2021). Similarly, Zhang et al. (2016b) showed that the application of PHB-producing *Trichoderma longibrachiatum* T6 increased wheat growth and salt stress tolerance. The authors suggested that PHB might play a role in the plant growth-promoting effects of certain microorganisms, possibly by enhancing the colonization of the plant roots and improving nutrient availability.

PHB and Microbial Interactions

Recent studies have revealed that PHB can also play a crucial role in mediating microbial interactions. For instance, PHB has been shown to influence quorum sensing, a cell-to-cell communication system employed by bacteria to regulate gene expression in response to population density (Kalia et al., 2019). In *Pseudomonas aeruginosa*, PHB degradation products, such as 3-hydroxybutyrate (3HB), modulate the production of virulence factors and biofilm formation through quorum sensing (Boyd & Chakrabarty, 1994; Ochsner et al., 1994). Moreover, PHB-producing microorganisms, such as certain strains of *Trichoderma spp*., can exhibit plant growth-promoting properties, suggesting that PHB might be involved in the beneficial interactions between these microorganisms and plants (Zhang et al., 2016a). In this context, PHB could act as a signalling molecule, mediating the crosstalk between microorganisms and their environment, including other microbes and host plants.

1.2 Project Objective

The objective of this project is to investigate the effects of *Trichoderma afroharzianum* T-22, *Bacillus amyloliquefaciens*, and poly-β-hydroxybutyrate (PHB) biostimulant firstly on potentially tackling the challenges of solid digestate, and secondly on the growth, stress tolerance, nutrient uptake, biomass and fruit yield of tomato plants cultivated in a solid digestate, peat, and pumice substrate. This substrate can offer a sustainable and environmentally friendly approach to organic tomato cultivation. By reducing the proportion of peat and incorporating pumice and solid digestate, the negative environmental impacts associated with peat extraction can be mitigated, while still benefiting from the desirable properties of peat. The addition of plant growth-promoting microorganisms, such as *Trichoderma afroharzianum T-22* and *Bacillus amyloliquefaciens*, and the application of poly-β-hydroxybutyrate (PHB) to this substrate blend can further

enhance tomato plant growth and resilience to stress (Harman et al., 2004; Chowdhury et al., 2015b). The aim is to determine the potential synergistic effects of these plant growth-promoting agents and solid digestate and assess their applicability as sustainable alternatives to conventional agricultural practices for enhancing tomato production.

1.2.1 Research Questions

- Does the combined application of *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens* in a solid digestate, peat, and pumice substrate enhance tomato plant growth compared to single applications or control treatments?

- Does the application of PHB biostimulant in combination with *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens* lead to synergistic effects on tomato plant growth and stress tolerance?

- How does the application of *Trichoderma afroharzianum* T-22, *Bacillus amyloliquefaciens*, and PHB biostimulant affect the nutrient uptake and nutrient use efficiency of tomato plants grown in a solid digestate, peat, and pumice substrate?

- What are the effects of different treatments on the overall fruit yield, chlorophyll content, photosynthesis, leaf area, and the biomass of tomato plants?

1.2.2 Hypotheses

- The combined application of *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens* will result in higher tomato plant growth compared to single applications or control treatments.

- The application of PHB biostimulant in combination with *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens* will lead to synergistic effects on chlorophyll, photosynthesis, and stress tolerance.

- The different treatments will have varying effects on the nutrient uptake and nutrient use efficiency of tomato plants, with the combined application of microorganisms and PHB biostimulant potentially showing the highest efficiency.

- Tomato plants treated with the combined application of *Trichoderma afroharzianum* T-22, *Bacillus amyloliquefaciens*, and PHB biostimulant will result in better fruit yield compared to the other treatments.

2. Materials and Methods

The experimental design consisted of six treatments, each containing ten tomato plants (*Solanum lycopersicum L.*), the variety of which was Flavorino F1, obtained from Olssons Frö AB. All treatments used the same substrate, composed of solid digestate, peat, and pumice in a ratio of 2:1:1, with additional variables applied as follows:

1. Control: No additional treatments were applied to the plants in this group.

2. *Trichoderma afroharzianum* T-22 treatment: Tomato roots were inoculated with *Trichoderma afroharzianum* T-22 only.

3. *Bacillus amyloliquefaciens* treatment: Tomato roots were inoculated with *Bacillus amyloliquefaciens* only.

4. Combined microbial treatment: Tomato roots were inoculated with both *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*.

5. Combined microbial and PHB biostimulant treatment: Tomato roots were inoculated with both *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*, along with seed, root, and foliar application of the commercial PHB biostimulant, Albit.

6. PHB biostimulant treatment: Tomato plants received seed, root, and foliar application of the commercial PHB biostimulant Albit only.

The greenhouse experiment was conducted on a table, under 4 high-pressure sodium (HPS) lamps, operating from 07:00 to 21:00 daily. Temperature and ventilation settings were maintained at a minimum of 20°C during the day and 18°C at night, with ventilation temperatures set at 22°C during the day and 20°C at night. Humidity was maintained at 65%, and shading was activated when light intensity reached 400 W/m^2.

A drip irrigation system was installed, with water delivery starting at 250 mL per plant per day and gradually increasing to 2 litres per plant per day during the final two weeks of the experiment. Initial EC of the substrate was 0,7mS and pH was 8,4 due to the high pH of the solid digestate (8,8). In order to reduce pH value in the substrate, droplets of sulfuric acid were added in the irrigation barrel of the cultivation, until the substrate's pH was reduced to 6.5. This setup aimed to evaluate the impact of the various treatments on tomato plant performance and yield under controlled environmental conditions, providing valuable insights into the potential benefits of microbial inoculations and biostimulants for sustainable agriculture.

2.1 Root Inoculation

Trichoderma afroharzianum T-22 and *Bacillus amyloliquefaciens* used in this study, were obtained from a reputable culture collection. For *T. afroharzianum* T-22, potato dextrose agar petri dishes with developed mycelium, were harvested by adding sterile distilled H2O, scrapping their surface, and filtered through sterile cotton. For *B. amlyloliquefaciens*, a single colony was taken out from a pure culture and incubated in tryptic soy broth for 48h at 25 C. After the extracting process with 0,85% NaCl, while centrifuged at 3000 rpm for 15min, sterile tap H_2O was added. The final suspension was then vortexed to ensure a uniform distribution of the microorganism.

Prior to root inoculation, the microorganism suspensions were prepared by transferring the *T. afroharzianum T-22* and *B. amlyloliquefaciens* suspensions to sterile test tubes in the amount of 1ml. Seeds were germinated in vermiculite at 21 degrees C. 15 days after germination, their roots were exposed by gently removing the vermiculite around the root system. The roots were then immersed in the respective microbial suspensions for approximately 60 minutes, ensuring that the roots were thoroughly coated with the suspension. Following inoculation, the seedlings were transplanted to their pots and the substrate was carefully packed around the roots to prevent any damage. The pots were placed in the greenhouse chamber under controlled conditions of 21 degrees C and 65% humidity, to monitor their development over time.

2.2 Root Re-inoculation Throughout the Cultivation Period

To ensure the presence of the inoculated microorganisms, *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*, throughout the cultivation

period, a root re-inoculation process was implemented for the different treatment groups.

For the T-22-only treatment, a suspension of 10 mL of T-22 in sterile distilled H_2O was prepared. This suspension was carefully poured directly above the roots of the plants in this treatment group.

In the case of the *Bacillus amyloliquefaciens*-only treatment, a suspension of 10 mL of *Bacillus amyloliquefaciens* in sterile tap H_2O was prepared. This suspension was similarly applied to the roots of the plants in this treatment group, allowing for the even distribution of the microorganism throughout the root system.

For the combined treatment group, where both microorganisms were applied, a mixture of 5 mL of the T-22 suspension and 5 mL of the *Bacillus amyloliquefaciens* suspension was prepared. This combined suspension was then applied to the roots of the plants in this treatment group, providing an equal distribution of both microorganisms to the root system.

The root re-inoculation process was repeated at regular intervals 3 times throughout the cultivation period.

2.3 Application of Albit

Albit was applied to the tomato plants to evaluate its potential effects on plant growth, health, and productivity. The application process consisted of a series of treatments, including seed inoculation, foliar applications, and root applications.

The initial Albit application started with seed inoculation. Tomato seeds were placed in an Albit suspension consisting of 2 mL of Albit per litre of distilled H_2O. The seeds were stirred gently in the suspension for 3 hours, ensuring thorough coverage of the biostimulant.

After transplanting the seedlings into their main pots, the first foliar application of Albit was carried out immediately. A second foliar application was performed one month later. Both applications took place according to recommendations of the biostimulant's manufacturer. For these foliar treatments, a sprayer was used to apply a mixture of 2 mL of Albit in 10 L of H_2O, with approximately 50 mL of the mixture sprayed onto each plant, ensuring even distribution across the foliage.

A root application of Albit was conducted 10 days after the second foliar application. For this treatment, 50 mL of the aforementioned Albit mixture (2 mL of Albit in 10 L of H_2O) was applied directly to the roots of each plant. Finally, a

third foliar application of Albit was performed 10 days after the root application, using the same method and mixture as the previous foliar treatments.

The combination of seed inoculation, foliar applications, and root applications of Albit aimed to provide the tomato plants with a comprehensive exposure to the biostimulant.

2.4 Lysimeter Installation and Nutrient Analysis

Lysimeters, equipped with vacuum tubes, were utilized to collect solution from the tomato plant substrates. To begin the process, a lysimeter was carefully installed in each pot, ensuring minimal disturbance to the roots and substrate. The vacuum tubes were then attached to the lysimeters, allowing for efficient extraction of the liquid samples.

Following installation, the lysimeters were left undisturbed for a period of 24 hours. This duration enabled sufficient contact time between the lysimeters and the substrate. After 24 hours, the liquid samples were extracted from the lysimeters using the attached vacuum tubes.

The nutrient analysis focused on the measurement of ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and phosphate-phosphorus (PO_4-P) concentrations. HACH LANGE kits were employed for this purpose, following the manufacturer's instructions for each respective nutrient test. For NH_4-N, the kit LCK303 was used, for NO_3-N, the kit LCK340, and for PO_4-P, the kit LCK349 The measurements were conducted 3 times, one in the first days after transplanting, another in the middle of the cultivation period, and the last one a day before harvesting.

2.5 Tomato Plant Biomass Assessment

To evaluate the impact of fungal and bacterial inoculation, as well as Albit, on plant growth, the biomass of the tomato plants was assessed at the end of the experiment. The plants were carefully removed from their pots, taking care not to damage the roots or the aerial parts.

The aerial portions were separately placed into pre-weighed aluminium foil packages, which were then sealed tightly to prevent contamination or moisture exchange. These packages were transferred to a drying chamber maintained at a constant temperature of 105°C for a period of 3 to 7 days. This temperature and duration ensured complete removal of moisture from the plant tissues without

causing damage or decomposition. The dried aerial portions were then carefully weighed using a high-precision analytical balance.

2.6 Leaf Area Measurements

Leaf area measurements were conducted to assess the impact of fungal and bacterial inoculation, as well as Albit, on the overall leaf development of the tomato plants.

To measure the leaf area, all leaves were carefully detached from each tomato plant, ensuring minimal damage to the leaf tissues. A leaf area meter (LI-3100C Area Meter, LI-COR Biosciences) was used for the measurements, following the manufacturer's instructions.

2.7 Chlorophyll Measurements

Chlorophyll content was assessed weekly over an 8-week period. Two leaves per plant were selected for the measurements to provide a statistically robust representation of each plant's chlorophyll content. The Apogee Instruments MC-100 Chlorophyll Concentration Meter was employed for this purpose, following the manufacturer's guidelines.

At each weekly time point, two fully expanded, healthy leaves from each plant were chosen for the measurements. These leaves were preferably from the same node to ensure similar light exposure and developmental stage. The average chlorophyll content for the two leaves was then calculated, providing a representative value for each plant at that particular time point. The MC-100 Chlorophyll Concentration Meter was calibrated according to the manufacturer's instructions before each measurement session. This process was repeated every week for 8 weeks.

2.8 Photosynthesis Measurements

To evaluate the impact of fungal and bacterial inoculation on the photosynthetic performance of the tomato plants, photosynthesis measurements were conducted using the ADC BioScientific LCpro Photosynthesis System. Six measurements per treatment were taken for statistical purposes, with two measurements conducted on each plant at different time intervals to observe the differences in stomatal activity.

On the day of measurements, the LCpro device was calibrated according to the manufacturer's instructions to ensure accurate readings. The LCpro LED lamp was

also set up to provide a steady light condition for each measurement, ensuring uniformity across all measurements.

The first measurement session was conducted between 09:00 to 12:00h, targeting the morning peak of stomatal activity. A fully expanded, healthy leaf from each plant was selected for the measurement, preferably at a similar node position across all plants. The leaf chamber of the LCpro device was carefully clamped onto the selected leaf, ensuring a proper seal and avoiding any damage to the leaf tissue. The LED lamp was positioned at an appropriate distance from the leaf chamber to provide the required light intensity at 200. The device was then activated, and photosynthesis measurements were recorded as net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}). Once the first measurement was completed, the leaf chamber was removed, and the same procedure was repeated for the other plants within the treatment group.

The second measurement session took place between 13:30 to 17:00 h to capture the stomatal activity during the afternoon period. The same leaves were used for these measurements to maintain consistency.

2.9 Re-isolation of *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*

To confirm the presence of the inoculated microorganisms, *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*, in the plant substrate, re-isolation was performed three times during the project period. For each sampling event, substrate samples were collected from each pot.

A 1 g sample of substrate was mixed with 10 mL of 0.85% NaCl solution and stirred for 30 minutes to achieve a homogenous suspension. Subsequently, a series of tenfold dilutions from 10^{-1} to 10^{-8} were prepared by transferring 1 mL of the initial suspension to 9 mL of 0.85% NaCl solution in a stepwise manner.

For the re-isolation of *Trichoderma afroharzianum* T-22, TSM (*Trichoderma* Selective Medium) agar plates were prepared, supplemented with antibiotics streptomycin and tetracycline to inhibit bacterial growth. For the re-isolation of *Bacillus amyloliquefaciens*, Nutrient Agar plates supplemented with streptomycin were used.

Two replicates from each dilution step of the dilution series for both microorganisms were plated on the appropriate agar plates. Sterile glass beads were used to evenly distribute the suspensions on the agar surface. The plates were then

incubated at their respective optimal temperatures, as per the requirements of the individual microorganisms.

Colonies were counted after 24 hours of incubation in 25 degrees C for *Bacillus amyloliquefaciens* and after 72 hours in 25 degrees C for *Trichoderma afroharzianum* T-22.

2.10 Yield Assessment

To evaluate the effects of the fungal and bacterial inoculation on the productivity of the tomato plants, a comprehensive yield assessment was carried out. All tomato fruits were harvested on a predetermined day, regardless of their maturity stage or size, to provide a consistent basis for comparison.

Following the harvest, each tomato fruit was measured for weight. The weight of each fruit was determined using a high-precision analytical balance, ensuring accuracy in the recorded values. These measurements were recorded for each individual fruit.

The collected data on fruit weight were used to calculate the overall yield for each plant, as well as to assess any potential differences in fruit morphology between the inoculated and control plants.

2.11 Statistical Analyses

To evaluate the significance of the observed effects and to identify any meaningful patterns or relationships in the data, statistical analyses were performed on the collected results. All data were input into Microsoft Office Excel, which was used to organize, manage, and analyze the data. Graphs and tables were created in Excel to visually represent the data and facilitate the identification of trends and patterns. Descriptive statistics, such as mean, standard deviation, and range, were calculated to summarize the central tendency and variability of the data for each variable.

To determine whether there were significant differences between the treatment groups, inferential statistical tests were conducted. Analysis of variance (ANOVA) was used to compare the means of multiple groups, testing the null hypothesis that there were no significant differences between the group means. When significant differences were detected by ANOVA, post-hoc tests, such as Tukey's HSD, were performed to identify which specific groups differed significantly from each other. The software used for analysis of variance (ANOVA) was Minitab.

3. Results

3.1 Biomass assessment

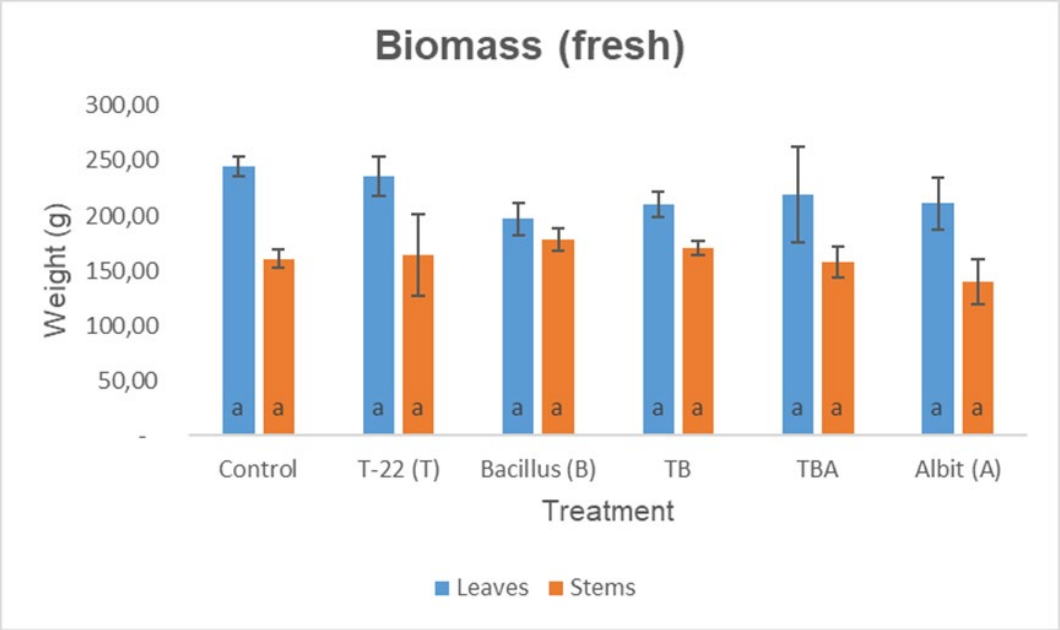

Figure 1 Fresh weight of leaves and stems. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment. Different letters denote significant differences at p-value < 0.05.

The biomass assessment for fresh weight did not show any significant differences between the treatments (figure 1). For fresh biomass, the Control treatment achieved the highest fresh leaf biomass, followed by *T. afroharzianum* T22 only treatment, while *B. amyloliquefaciens* alone treatment had the lowest. In contrast, *B. amyloliquefaciens* had the highest fresh stem biomass, and the lowest was demonstrated by Albit (PHB) alone. When evaluating the dry leaf biomass, the control treatment demonstrated the highest biomass production, while the *B. amyloliquefaciens* -only treatment displayed the lowest. In the case of dry stems,

however, *B. amyloliquefaciens* combined with *T. afroharzianum* T-22 showed the highest biomass, whereas Albit (PHB) alone showed the lowest (figure 2).

These trends suggest that the different treatments might have influenced the distribution of biomass across different plant parts. Nevertheless, they did not lead to significant overall changes in total plant biomass, as the results were not statistically significant.

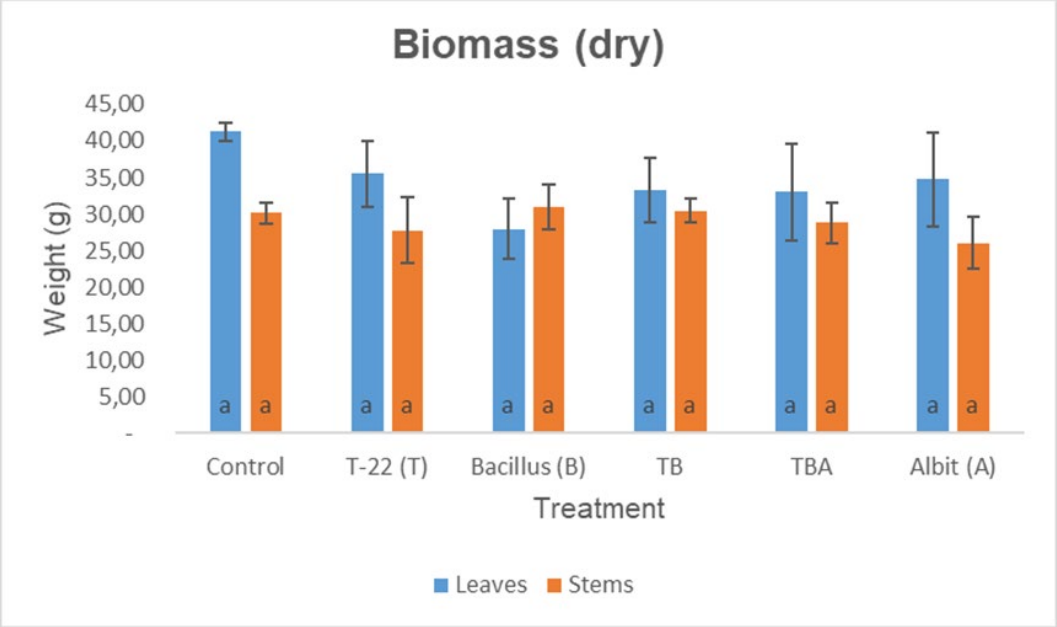

Figure 2 Dry weight of leaves and stems. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment. Different letters denote significant differences at p-value < 0.05.

3.2 Chlorophyll

In the assessment of chlorophyll content, a general trend emerged, revealing the Albit (PHB) treatment as the most successful in promoting chlorophyll synthesis, followed by *T. afroharzianum* T-22, the combination of *T. afroharzianum* T-22 and *B. amyloliquefaciens* , the combination of all three, and finally *B. amyloliquefaciens* . The control group had the lowest overall chlorophyll content (figure 3).

On March 10, significant differences were observed between *B. amyloliquefaciens* and Albit (PHB) (p= 0.004), as well as between the control and Albit (PHB) with a p-value of 0, and *T. afroharzianum* T-22 and Albit (PHB) (p= 0.013). In all three observations, the Albit treatment had the highest chlorophyll content.

On March 15, significant differences were found between the combination of *T. afroharzianum* T-22 and *B. amyloliquefaciens (TB),* and Albit (PHB) with a (p= 0.007), the control and Albit (PHB) (p= 0.024), and *T. afroharzianum* T-22 and Albit (PHB) (p= 0.066 and 0.07, respectively). Similarly, the Albit treatment resulted in the highest chlorophyll cintent in comparison with the aforementioned treatments.

On April 13, *T. afroharzianum* T-22 was significantly different than the control (p= 0.011), and also differed slightly from the combination of all three treatments (TBA) (p= 0.07), showing the highest chlorophyll content value.

On April 20, a significant difference was found between the combination of *T. afroharzianum* T-22 and *B. amyloliquefaciens* (TB) and the control (p= 0.029), as the control treatment had the highest chlorophyll content.

There were no significant differences observed on 23/3/2023, 30/3/2023, 6/4/2023, and 27/4/2023, however the measurement of 30th of March was the point that each treatment had its highest value, and from that point after, chlorophyll content demonstrated an ongoing reduction in almost every treatment.

This analysis indicates that the Albit (PHB) treatment generally resulted in higher chlorophyll content in tomato plants compared to the other treatments, especially during the initial stages of cultivation.

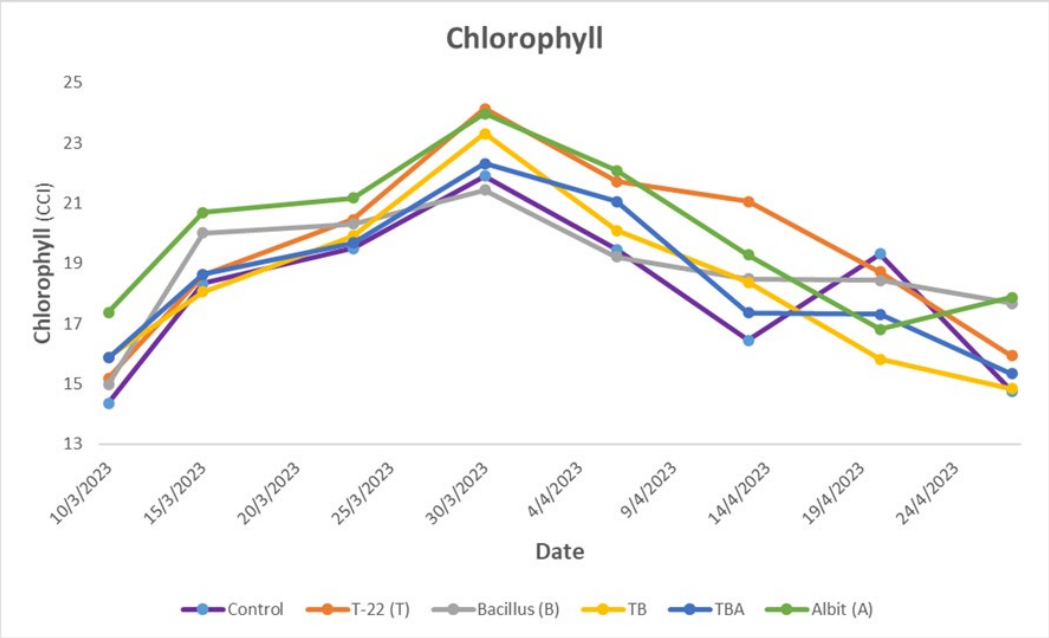

Figure 3 Chlorophyll content of the tomato plants of each treatment, represented in 8 different measurements, one per week of cultivation. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA),

and 6) Albit (PHB (A)). Measurements were taken from all plants. Significant differences at p-value < 0.05.

3.3 Photosynthesis

In terms of photosynthesis, the general trend observed was that the plants under the control treatment had a lower absorbance (mmol m^{-2} s^{-1}) compared to those treated with *B. amyloliquefaciens*, which in turn were lower than those treated with *T. afroharzianum* T-22. The plants treated with the combinantion of all three - *T. afroharzianum* T-22, *B. amyloliquefaciens*, and Albit (PHB) - resulted in the highest absorbance rates (figure 4).

Regarding stomatal conductance (mmol m^{-2} s^{-1}), the control group displayed the lowest values, followed by Albit (PHB), *B. amyloliquefaciens* , the combined treatment of *T. afroharzianum* T-22 and *B. amyloliquefaciens* , *T. afroharzianum* T-22 individually, and the highest values were observed in the all-combined treatment group (figure 5).

Despite these observable trends in both absorbance and stomatal conductance, no significant differences were detected among the treatments.

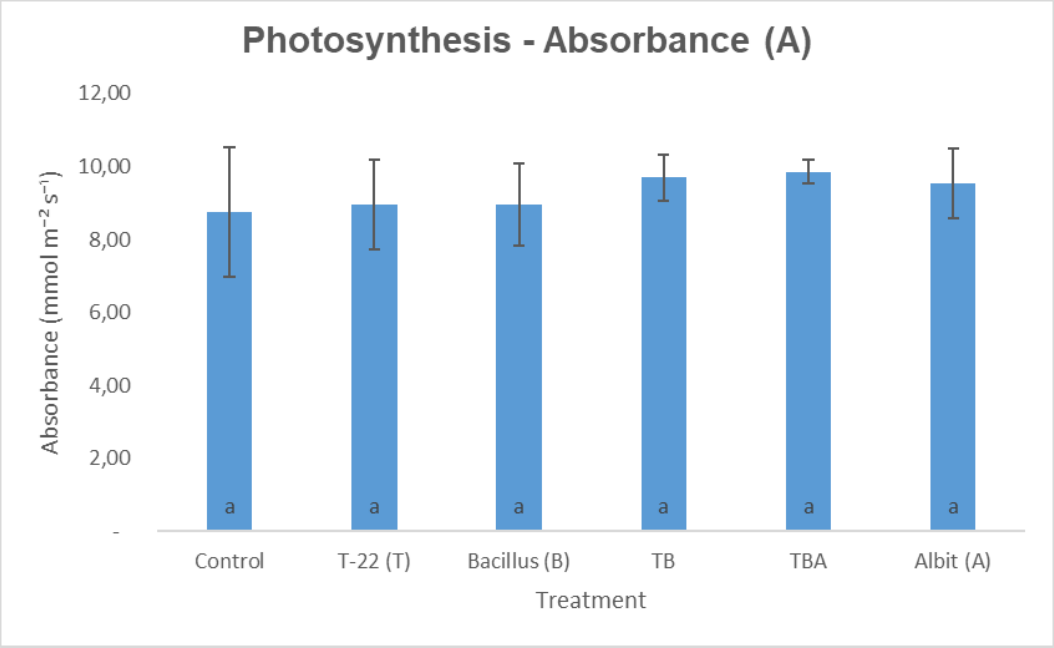

Figure 4 Absorbance rate of the tomato plants of each treatment, representing photosynthesis. Measurements were taken in the middle of the cultivation period. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB)

(TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and 2 measurements per plant. Different letters denote significant differences at p-value < 0.05.

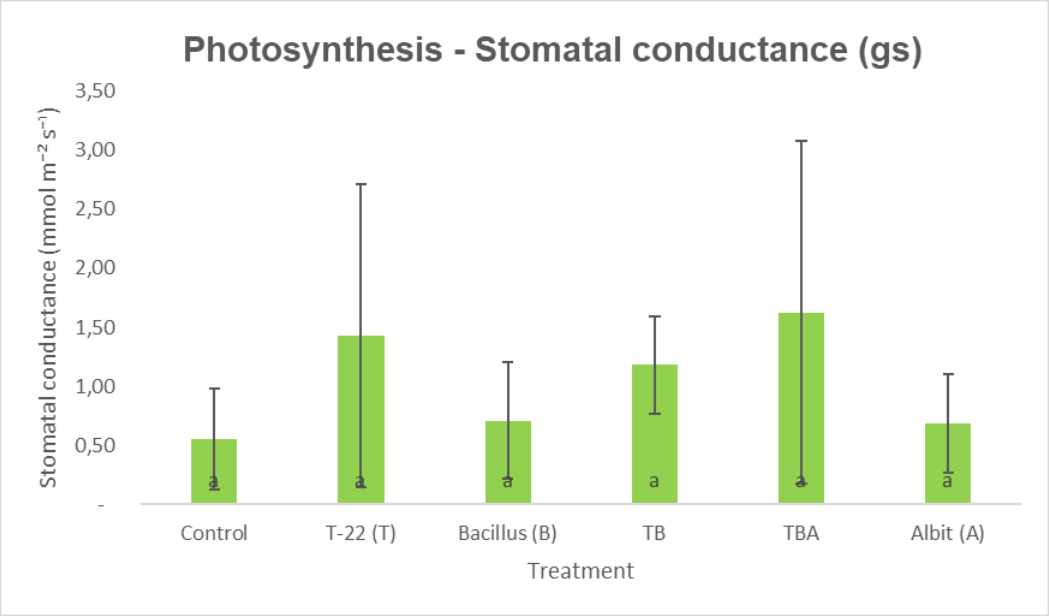

Figure 5 Stomatal conductance (gs) of the tomato plants of each treatment, representing photosynthesis. Measurements were taken in the middle of the cultivation period Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and 2 measurements per plant. Different letters denote significant differences at p-value < 0.05.

3.4 Leaf area

Regarding the leaf area measurements, tomato plants treated with the control treatment demonstrated the largest leaf area. This was closely followed by plants treated with *T. afroharzianum* T-22 alone. Tomato plants treated with Albit (PHB) exhibited the smallest leaf area. Despite these observed trends, it should be noted that the differences between the treatments were not statistically significant (figure 6).

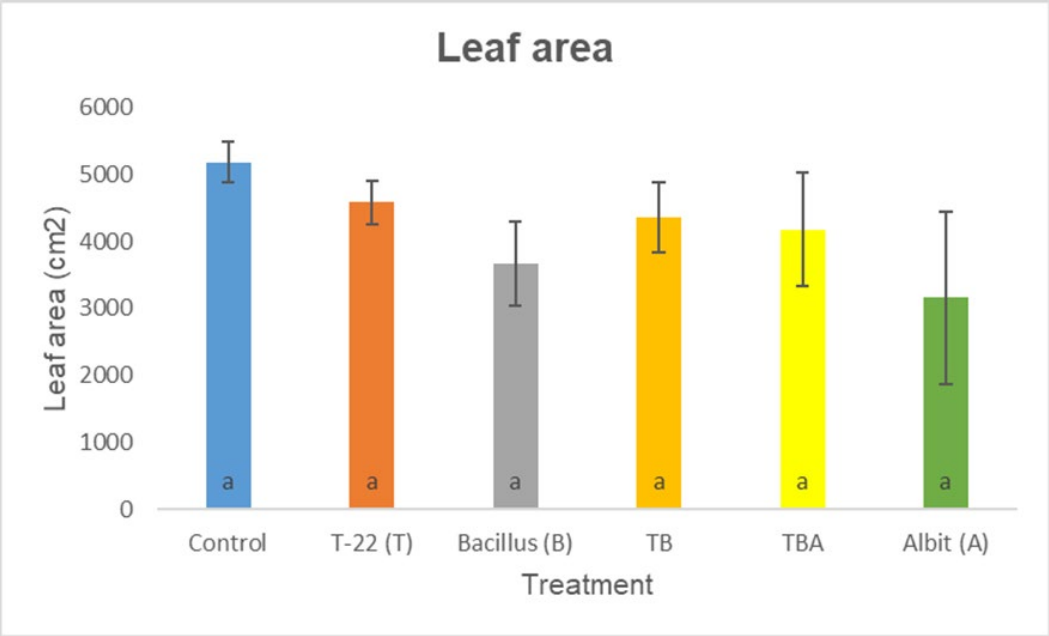

Figure 6 Leaf area (cm2) of the tomato plants of each treatment. Measurements were taken at the end of the cultivation period Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment. Different letters denote significant differences at p-value < 0.05.

3.5 Substrate Nutrient analyses

For the substrate nutrient analyses, in the middle of cultivation, the trends were as follows: the highest concentration of NO_3-N(mg/ml) was observed in plants treated with *B. amyloliquefaciens*, whereas the lowest was in the control treatment (figure 7). For NH_4-N(mg/ml), the highest concentration was noted in the Albit (PHB) treatment, while the lowest concentration was found in the treatment combining *T. afroharzianum* T-22, *B. amyloliquefaciens* and Albit (PHB) (figure 8). Lastly, the highest PO_4-P(mg/ml) concentrations were detected in plants treated with *B. amyloliquefaciens*, and the lowest in the control treatment (figure 9). Nevertheless, it is crucial to note that there were no statistically significant differences in these nutrient concentrations across treatments.

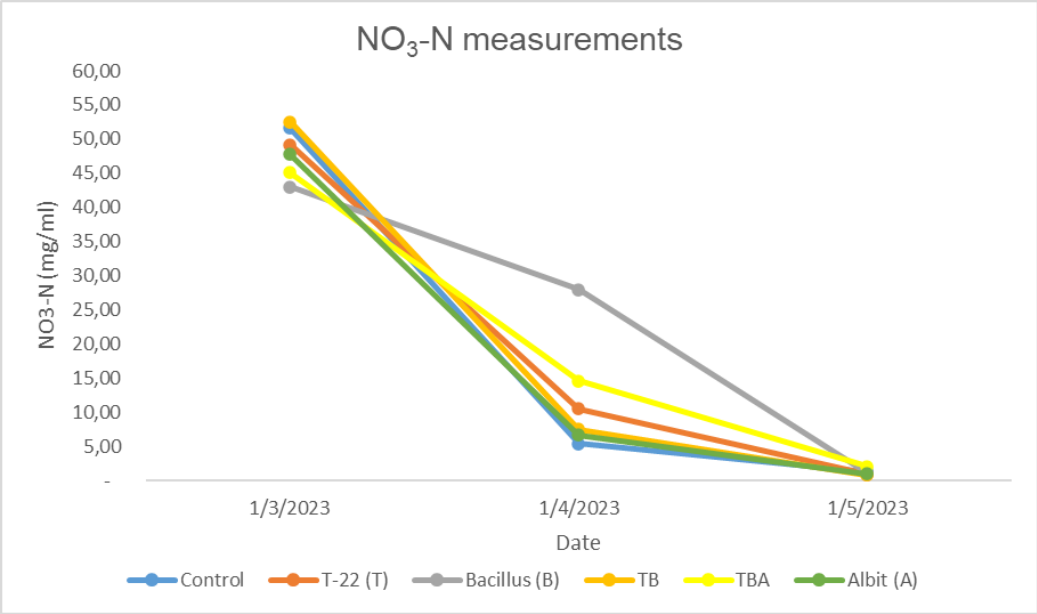

Figure 7 NO_3-N (mg/ml) of the tomato plants' substrate of each treatment. Three measurements were taken, the first two weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and one sample per plant was analyzed each time. Significant differences at p-value < 0.05. Extended data are shown in Appendix 1 (Table2)

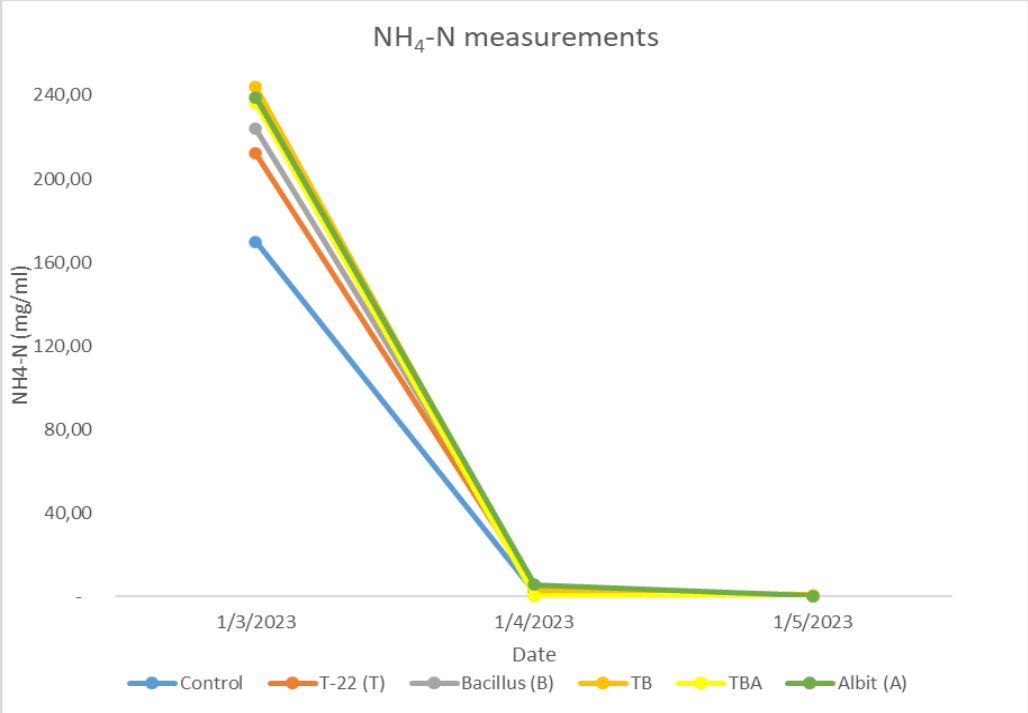

Figure 8 NH_4-N (mg/ml) of the tomato plants' substrate of each treatment. Three measurements were taken, the first two weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the figure are:

1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and one sample per plant was analyzed each time. Significant differences at p-value < 0.05. Extended data are shown in Appendix 1 (Table3)

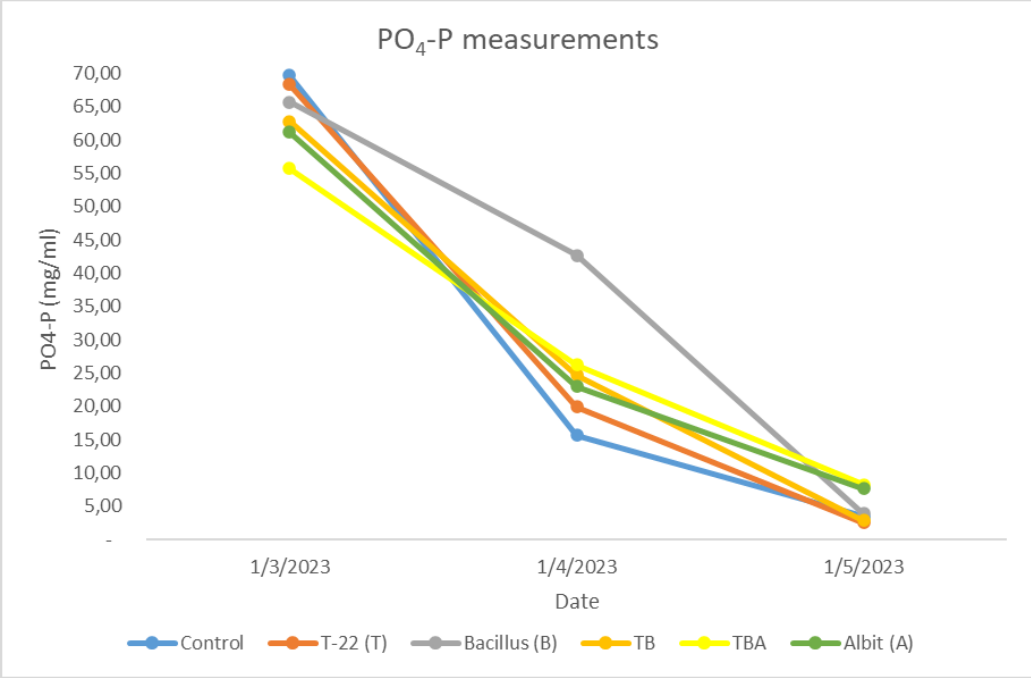

Figure 9 PO_4-P (mg/ml) of the tomato plants' substrate of each treatment. Three measurements were taken, the first two weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and one sample per plant was analyzed each time. Significant differences at p-value < 0.05. Extended data are shown in Appendix 1 (Table4)

3.6 Flowers and Fruits

In terms of the number of flowers, a notable statistical difference(p=0,04) emerged with the Albit (PHB) treatment resulted in the highest flower count in comparison with the control treatment that showed the lowest count. Despite this pattern, it is essential to highlight that the differences between the rest of the treatments were not statistically significant. In terms of fruit production, the Albit (PHB) treatment outperformed the others, producing the most fruit, while *B. amyloliquefaciens* treatment produced the least (figure 10). Regarding both the fresh and dry weight of the fruits, the highest weights recorded in the control treatment and the lowest in

the *B. amyloliquefaciens* treatment. However, similarly to the flower count, these differences in fruit production and weight were not statistically significant (figures 11 & 12).

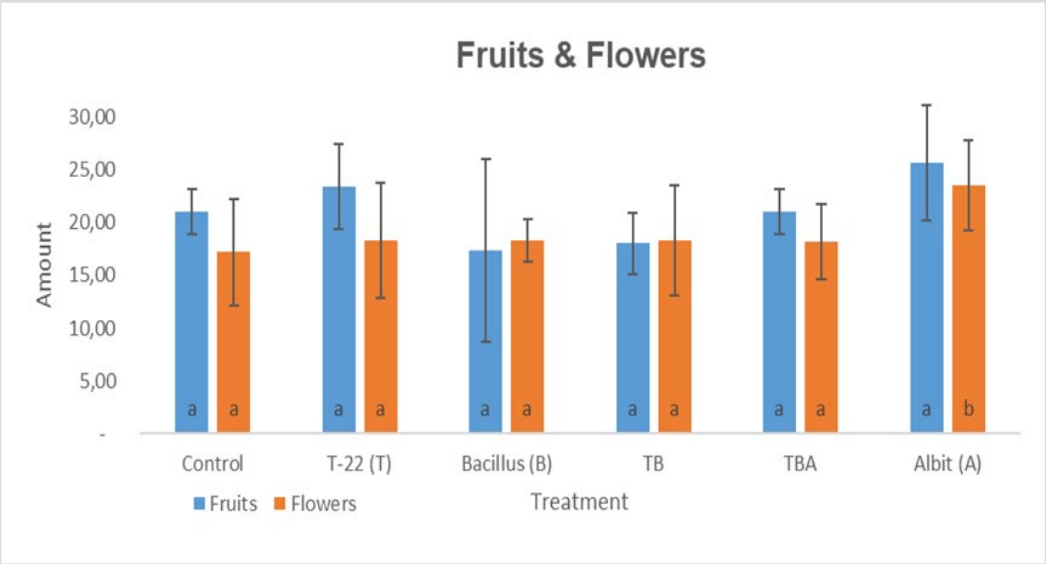

Figure 10 Amount of flowers and fruits of the tomato plants of each treatment. Measurements for flowers were taken the first week after the first flower opened. Measurements of the fruit amount took place during harvesting. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment. Different letters denote significant differences at p-value < 0.05.

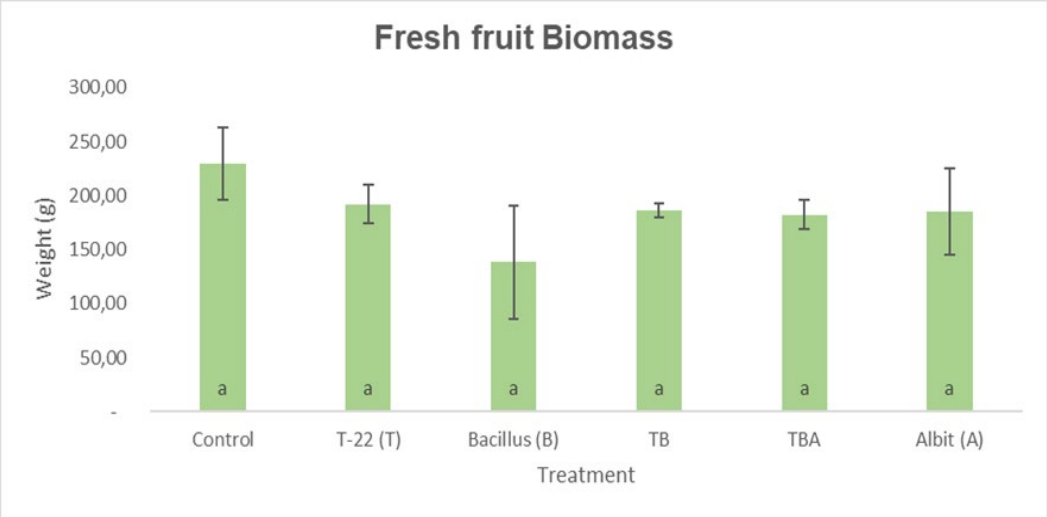

Figure 11 Fresh fruit biomass (g) of the tomato plants of each treatment. Measurements were taken at the end of the cultivation period. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment. Different letters denote significant differences at p-value < 0.05.

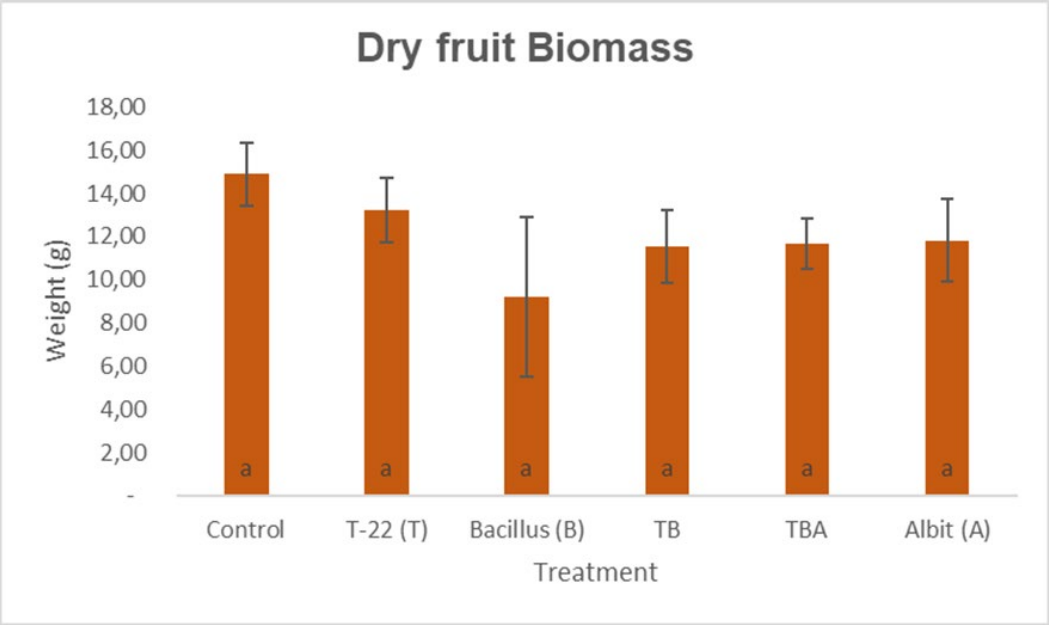

Figure 12 Dry fruit biomass (g) of the tomato plants of each treatment. Measurements were taken 5 days after drying in 105 °C. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment. Different letters denote significant differences at p-value < 0.05.

3.7 Final full nutrient analysis

Upon conducting a comprehensive final nutrient analysis, the following observations were made (table 1). In terms of Magnesium (Mg) content, the Albit (PHB) treatment had significantly lower concentrations than the control treatment (P=0.02). Likewise, the *T. afroharzianum* T-22 *B. amyloliquefaciens* Albit (PHB) combined (TBA) treatment showed significantly lower Mg levels than the control treatment (P=0.047).

When considering Manganese (Mn) content, the Albit (PHB) and *B. amyloliquefaciens* treatments were both found to have significantly lower levels than the control treatment (P=0.009). Similarly, the Albit (PHB) and *B. amyloliquefaciens* treatments demonstrated significantly lower Mn levels than the *T. afroharzianum* T-22 treatment (P=0.042). Furthermore, the TBA treatment showed a significantly lower Mn concentration compared to the control treatment (P=0.012).

In the context of Sodium (Na) content, the *B. amyloliquefaciens* treatment presented the lowest levels. However, these differences were not statistically significant. Finally, the Phosphorus (P) content was lowest in the *B. amyloliquefaciens* and

TBA treatments. Despite this, these differences were also not statistically significant. All the rest results depicted in table 1, showed no significant differences.

Table 1 Nutrients found in the final nutrient substrate analysis per treatment, as well as their standard deviation (ST.DEV). Treatments illustrated in the table are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and one sample per plant was analyzed. Significant differences p-value < 0.05.

	Control		T-22 (T)		Bacillus (B)		TB		TBA		Albit (A)	
		ST.DEV ±		ST.DEV ±		ST.DEV ±		ST.DEV ±		ST.DEV ±		ST.DEV ±
pH	5,30	-	5,27	0,05	5,63	0,17	5,45	0,24	5,50	0,16	5,37	0,09
EC mS/cm	0,64	0,07	0,68	0,10	0,50	0,09	0,64	0,06	0,55	0,11	0,69	0,09
Nitrogen mg/l	5,47	1,61	5,30	2,01	4,69	2,81	4,67	2,02	3,43	2,24	3,00	1,82
Nitrate-N mg/l <	1,00	-	1,00	-	1,00	-	1,00	-	1,00	-	1,00	-
Ammonium-N mg/l	5,67	1,70	5,33	1,89	4,33	2,36	4,67	1,70	3,67	2,36	3,00	2,16
Phosphorus mg/l	64,33	3,09	62,67	8,65	46,33	8,96	56,33	3,68	46,00	5,66	45,33	12,50
Potassium mg/l	100,33	24,09	65,67	21,64	64,00	21,42	77,67	29,85	64,00	32,62	75,67	23,21
Magnesium mg/l	123,33	4,71	110,00	-	105,33	10,50	110,00	-	99,00	1,41	95,33	13,20
Sulfur mg/l	5,67	0,47	5,67	0,94	4,33	0,94	6,00	1,63	4,33	0,47	4,00	0,82
Calcium mg/l	190,00	8,16	163,33	4,71	193,33	28,67	173,33	4,71	173,33	9,43	160,00	16,33
Manganese mg/l	1,53	0,09	1,43	0,05	1,07	0,12	1,23	0,12	1,09	0,10	1,07	0,12
Boron mg/l	0,60	0,05	0,72	0,08	0,57	0,12	0,60	0,10	0,52	0,06	0,50	0,07
Iron mg/l	0,94	0,19	0,74	0,06	0,72	0,10	0,74	0,07	0,72	0,05	0,68	0,07
Sodium mg/l	91,67	4,92	98,67	9,39	76,67	9,53	99,67	22,07	79,67	8,18	83,00	1,63
Aluminium mg/l	1,10	0,14	1,00	-	1,00	-	1,00	-	1,00	-	1,00	-

3.8 Colony forming unit (CFU) count

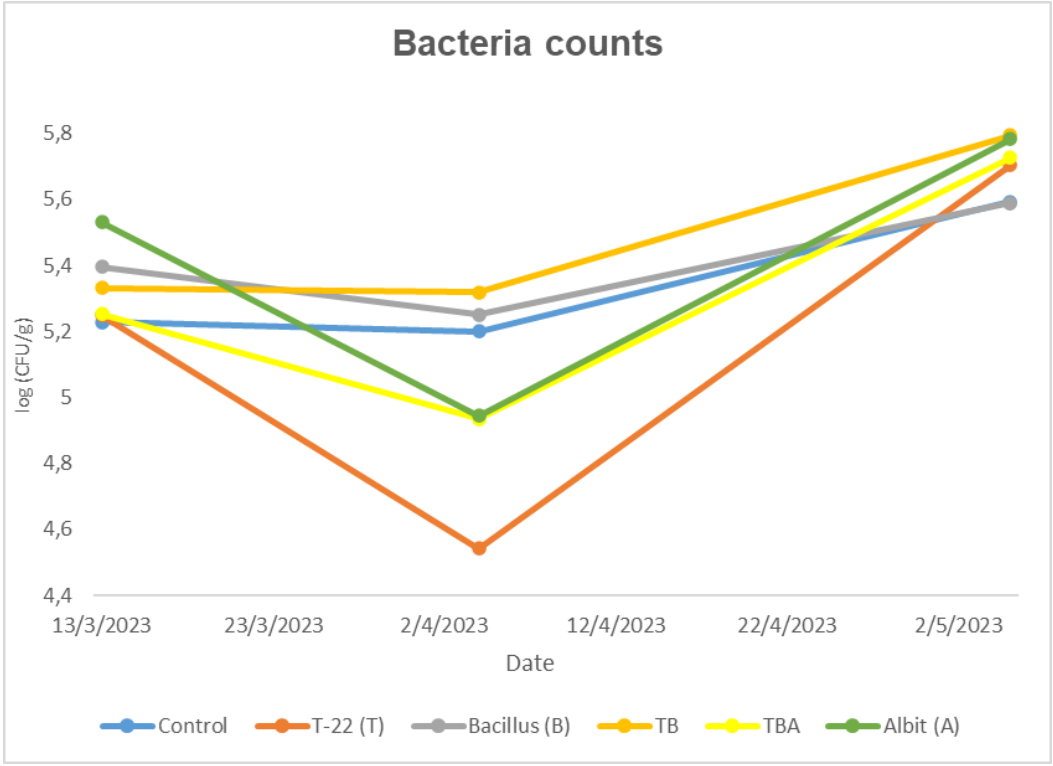

Figure 13 Bacteria counts found in the substrate of the tomato plants of each treatment. Three re-isolations took place, the first three weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and 2 replicate plates were used for each of the samples. Significant differences at p-value < 0.05.

Assessment of Colony Forming Units (CFU) count revealed intriguing trends over time. During the initial phase of cultivation, no significant differences were observed between the treatments in both bacterial and fungal CFU counts (figures 13 &14).

However, by the second re-isolation, *T. afroharzianum* T-22 (T) treatment resulted in a lower bacterial CFU count compared to the *B. amyloliquefaciens* (B) treatment, with this difference being statistically significant (P=0.046). Conversely, the *T. afroharzianum* T-22 *B. amyloliquefaciens* combined (TB) treatment showcased a higher bacterial CFU count than the T treatment, which also reached statistical significance (P=0.023).

By the third re-isolation, a shift was observed in the fungal CFU count. The *T. afroharzianum* T-22 *B. amyloliquefaciens* Albit (PHB) all-combined (TBA) treatment showed a significantly lower fungal CFU count than the A treatment (P=0.004). Furthermore, both T and TB treatments exhibited significantly lower fungal CFU counts than the B treatment (P=0.022 and P=0.001 respectively). Notably, TBA treatment displayed lower fungal CFU counts than both B and control (C) treatments, with these differences being highly significant (P=0 and P=0.002 respectively). The rest of the comparisons did not reveal any statistically significant differences.

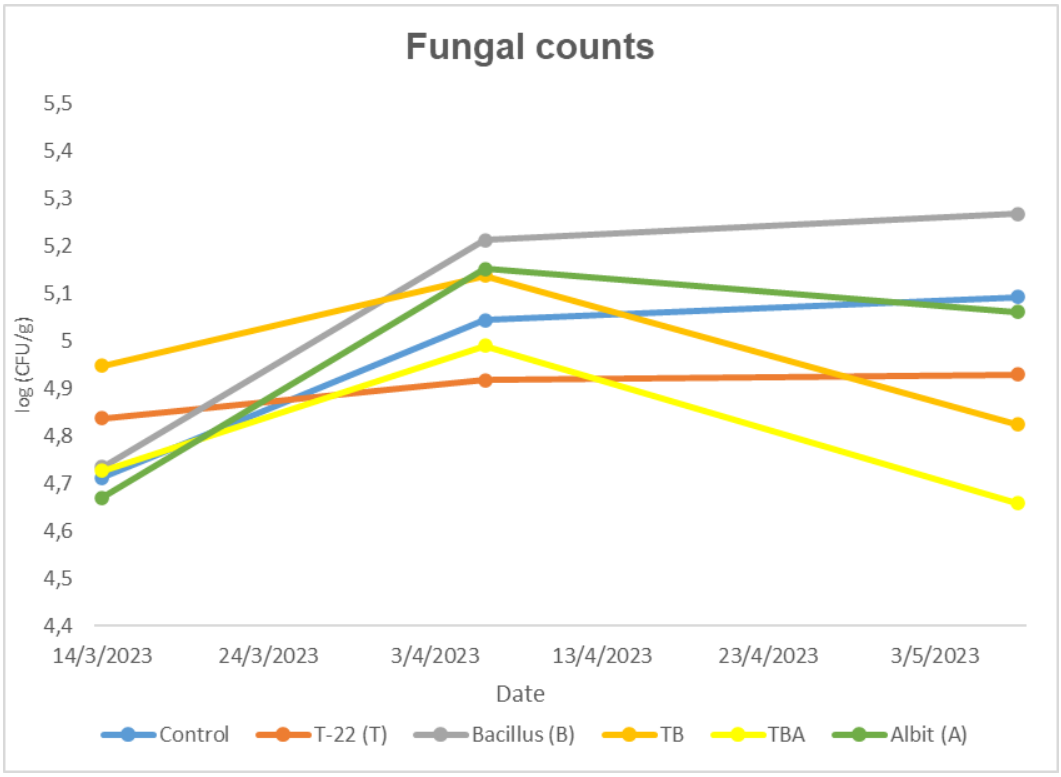

Figure 14 Fungal counts (Trichoderma spp), found in the substrate of the tomato plants of each treatment. Three re-isolations took place, the first three weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the figure are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants were used per treatment and 2 replicate plates were used for each of the samples. Significant differences at p-value < 0.05.

4. Discussion

The management of solid digestate, a by-product of biogas production, poses a substantial challenge to modern agriculture due to its high pH and potential nutrient unavailability for plant growth. The present study endeavoured to explore the utility of solid digestate as a component of a growing substrate, further enhanced by the application of *T. afroharzianum* T-22, *B. amyloliquefaciens*, and poly-β-hydroxybutyrate (PHB) biostimulant in promoting the growth and development of tomato plants.

The novel findings from this study contribute to the broader understanding of how an integrative approach using biological agents and biostimulants can modify and improve the properties of solid digestate as a growth medium. Understanding these interactions is crucial, given the urgent need for sustainable soil amendments and alternatives to conventional fertilizers (Kamali et al., 2022). This approach aligns with the global push towards more sustainable, circular economies in agriculture, as it leverages waste products and biological processes to improve crop productivity.

These results are discussed with reference to previous studies, to provide a comprehensive understanding of the observed plant growth trends and the potential of solid digestate. Several parameters were evaluated in the current investigation, including biomass accumulation, chlorophyll content, photosynthesis, leaf area, substrate nutrient analysis, flower and fruit yield, and final nutrient analysis. The occurrence and count of bacteria and fungi, essential for nutrient cycling and plant growth promotion, were also assessed.

4.1 Substrate and Treatment Effects on Plant Growth and Productivity

Understanding the impact of the experimental substrate and various treatments on plant growth is fundamental to discerning the potential application of these strategies in horticultural practices.

While there were no significant differences in biomass production among treatments, some discernible trends could be observed. The dry weight of leaves was highest in the control, followed by the *T. afroharzianum* T22 (T), Albit (PHB) (A), both microorganisms (TB), all-combined (TBA), and *B. amyloliquefaciens* (B) treatments. This could suggest that the control substrate, without any microbial inoculation or biostimulant application, was sufficient to support leaf growth. However, the growth-promoting effect of PHB and the beneficial microorganisms was evident in the higher biomass production compared to the *B. amyloliquefaciens*-only treatment. On the other hand, the *B. amyloliquefaciens*-only treatment led to the highest dry weight of stems, possibly due to the reported role of *Bacillus* spp. in promoting stem elongation (Miljaković et al., 2020). Those results did not comply with the hypothesis done prior to the experiment.

The chlorophyll content in the plants varied significantly over time among treatments. The highest chlorophyll content was consistently observed in the Albit (PHB) treatment, suggesting a stimulatory effect of PHB on chlorophyll synthesis. The combination of both beneficial microorganisms and PHB generally showed a positive trend on chlorophyll content, which proved the initial hypothesis, and can be linked to enhanced nutrient uptake, particularly nitrogen, an essential element for chlorophyll synthesis. Similarly, although the photosynthesis measurements did not yield significant differences among treatments, trends were observed. It appeared that PHB application promoted higher photosynthetic activity in the all-combined treatment (TBA), followed by the dual microorganisms (TB), *T. afroharzianum* T22, *B. amyloliquefaciens*, and control treatments. Given that photosynthesis is intrinsically linked to plant growth and productivity, the PHB and beneficial microorganisms could have potentially enhanced plant productivity by stimulating photosynthetic activity, and those results can also be linked to one of the hypotheses.

In terms of leaf area, the control treatment had the largest leaves, followed by *T. afroharzianum* T22, both microorganisms (TB), all-combined method (TBA), Bacillus amuloliquefaciens, and Albit (PHB) treatments. This may be an indication that the control had on the one hand, sufficient nutrients for leaf growth, but on the other hand, the control plants may have slower nutrient uptake rate that the other tretaments. The smaller leaf area in *B. amyloliquefaciens* and PHB treatments could be due to the higher energy investment in other plant parts such as stems or roots.

The nutrient analysis of the substrate revealed fluctuating trends in the nutrient content over time. Despite the lack of significant differences, it appeared that the *B. amyloliquefaciens* treatment generally led to higher nitrate, ammonium, and phosphate levels in the substrate, especially in the middle of the cultivation period,

when the treatments had the more obvious differences. This may be associated with the reported capacity of *Bacillus* spp. to enhance nitrogen mineralization and phosphorus solubilization in the soil, which makes nitrogen and phosphorus more available for plant uptake (Kumar et al., 2015). Although the initial hypothesis was that the all-combined (TBA) treatment would be the most efficient regarding nutrient uptake, it was partly proven that the addition of microorganisms could enhance this ability of the plants. The PHB biostimulant application, in this project, seemed to present an improved trend in certain plant growth parameters, particularly in combination with microorganisms. Although the effects were not statistically significant, it resonates with existing literature suggesting similar biostimulants could enhance nutrient uptake and stress tolerance in plants, however, there is not specific literature about PHB.

Fruit yield is a direct measure of plant productivity. Although there were no significant differences among treatments, the PHB treatment exhibited a positive trend in fruit yield (both in terms of fruit numbers and weight). This suggests the potential role of PHB in enhancing tomato productivity, aligning with one of the hypotheses, and also with previous research demonstrating that biostimulants like PHB could increase fruit yield by improving nutrient uptake, enhancing stress tolerance, and modulating plant hormonal balance (Calvo et al., 2014). However, the control method had the best results regarding fruit weight, demonstrating the capability of solid digestate alone to enhance plant growth and productivity, although there were not any significant differences.

Lastly, it is crucial, for the understanding of the results, to note that measurements for fruit yield, biomass, as well as the last measurements for chlorophyll content, and nutrients in the substrate, took place at the end of the project. At that point, many nutrients had been almost depleted, and plants were affected the most from clopyralid, which had worsened the plants' condition in the entity of their aerial part.

4.2 Microbial Interactions and CFU Count

Understanding microbial interactions within the plant's rhizosphere is key to determining the impact of various treatments on plant growth. This section discusses the implications of the CFU/g calculations conducted during the study and how different treatments may have affected the microbial populations within the substrate.

Bacteria and fungi play vital roles in the soil ecosystem, participating in nutrient cycling, enhancing plant growth, and protecting plants against pathogens

(Bulgarelli et al., 2013). In this study, CFU/g calculations were performed to estimate the viable counts of bacteria and *Trichoderma spp*. (fungi) in the substrate. It was observed that treatments containing *B. amyloliquefaciens* (B and TB) had higher bacterial CFU counts during the second re-isolation, which may be a sign that the re-inoculations assist the microorganism to establish in the substrate, thus forming more colonies than in the rest of the treatments. In contrast, treatments containing *T. afroharzianum* T22 (T, TB, TBA) influenced *Trichoderma spp*. (fungi) CFU counts during the third re-isolation, as in those treatments resulted in the lowest CFU count. This was mainly not only due to the fact that the microorganism had also been established in those substrates, but was also depicted on the TSM plates, where larger colonies were formed, creating a more uniform mycelium (data not shown). Moreover, the dual inoculation treatment (TB) exhibited higher bacterial CFU count compared to the single inoculation with *T. afroharzianum* T22 (T), suggesting potential synergistic effects of *B. amyloliquefaciens* and *T. afroharzianum* T22 co-inoculation on bacterial proliferation. This could be linked to their complementary mechanisms in promoting plant growth and health as referred before. Interestingly, the CFU counts in the Albit (PHB) treatment remained comparable to the control, although they showed higher amounts of colonies in the middle of the cultivation period. This may indicate that PHB alone may not directly influence microbial population dynamics in the substrate and potentially requires the assistance of other substrate compounds. The potential indirect effects of PHB on microbial communities, such as mediating plant-microbe interactions or modifying soil physicochemical properties, warrant further investigation.

Comparing those results with existing literature, similar findings were obtained. For instance, several studies indicate that *Trichoderma* strains are effective in enhancing plant growth and productivity (Contreras-Cornejo et al., 2016). In this study, various strains of *Trichoderma* demonstrated a similar effect, exhibiting a general trend towards improving plant growth parameters. *Bacillus* spp, too, are well-documented for their plant growth-promoting effects, as well as their biological control (Borriss, 2011). The combination of both microorganisms showed a slightly better effect than when used individually, suggesting a possible synergistic effect, consistent with the findings of Poveda and Eugui (2022). Moreover, the findings concerning the microbial populations, specifically the CFU counts of Trichoderma spp. and general bacterial species, align with a general understanding of microbial dynamics in the rhizosphere.

4.3 Nutrient Analysis and Plant Uptake

Plant nutrition is a vital aspect of plant growth and development. Nutrient availability and efficient uptake can significantly influence plant productivity and health. In this study, various nutrient analyses were performed on the substrate, including NO_3-N, NH_4-N, and PO_4-P, followed by a final full nutrient analysis.
The middle cultivation trends in NO_3-N, NH_4-N, and PO_4-P showed variation across treatments. For instance, *B. amyloliquefaciens* alone treatment, resulted in the highest values in both NO_3-N and PO_4-P, which can be an indicator of its phosphorus solubilization and nitrogen mineralization properties. However, without significant differences, these trends might suggest subtle changes in nutrient dynamics in response to the treatments. Plants have a complex relationship with soil microbes, including *B. amyloliquefaciens* and *T. afroharzianum* T-22, which can influence nutrient availability and uptake (Compant et al., 2010). For instance, *Bacillus* spp. are known for their nitrogen-fixing abilities, phosphorus solubilisation, and production of siderophores that bind and transport iron, a crucial micronutrient (Ahemad & Kibret, 2014).

In the final full nutrient analysis, magnesium and manganese concentrations varied significantly across treatments. Specifically, the control (C) showed higher magnesium compared to the Albit (PHB) treatment and the all-combined treatment (TBA), implying that these treatments might have facilitated magnesium uptake by the plants. Similarly, manganese was highest in the control and lowest in the Albit (PHB), and *B. amyloliquefaciens* treatments, possibly due to enhanced plant uptake or microbial interactions. It is also worth noting that *B. amyloliquefaciens* treatments had the lowest sodium, which may suggest a beneficial action of Bacillus in mitigating salt stress in plants (Ramadoss et al., 2013).

Even though there were no significant differences in phosphorus content, lower levels were observed in *B. amyloliquefaciens* and all-combined (TBA) treatments. This suggests potential phosphorus solubilising properties of *B. amyloliquefaciens*, as supported by previous research (Rodríguez & Fraga, 1999).

Nutrient availability and uptake by plants in response to various treatments are complex, intertwined phenomena. While this study revealed certain trends in nutrient content under different treatments, it is consistent with the notion that plant-microbe interactions could affect nutrient dynamics in the rhizosphere (Richardson et al., 2009). In particular, the observation regarding *Bacillus's* potential role in reducing sodium and solubilizing phosphorus in the substrate deserves further exploration. Overall, the data indicate that the applied treatments, particularly microbial inoculations and biostimulant application, may have

influenced nutrient availability and uptake by the plants, potentially contributing to the observed plant growth trends.

4.4 Implications for Sustainable Agriculture

The results of this study have far-reaching implications for sustainable agriculture, particularly in the context of optimizing substrate use and harnessing the power of microbial interactions and biostimulants for improved crop productivity. The use of solid digestate, peat, and pumice as substrates, in combination with microbial inoculation and PHB application, opens new pathways for enhancing the sustainability of horticultural practices.

Solid digestate, derived from anaerobic digestion of organic wastes, has been suggested as a potentially valuable substrate for plant cultivation due to its high nutrient content (Nkoa, 2014). The utilization of solid digestate in this study reinforces its potential as a valuable, sustainable alternative to traditional synthetic fertilizers, which are often associated with environmental pollution and degradation. However, the challenge lies in managing its nutrient availability and balancing microbial interactions to ensure optimal plant growth, as suggested by our results.

The continued use of peat in horticulture is a significant concern due to the environmental implications associated with its extraction (Paoli et al., 2022). On the other hand, pumice, a volcanic rock, has been shown to be a sustainable, reusable growing medium with excellent water and nutrient retention properties (Pérez-Urrestarazu et al., 2019). The success of using peat and pumice, particularly in combination with solid digestate in this study, demonstrates the potential for these materials to replace peat in some applications, reducing the environmental footprint of horticultural practices.

The use of beneficial microorganisms such as *T. afroharzianum* T22 and *B. amyloliquefaciens*, as well as the PHB biostimulant, highlights the increasing interest in leveraging biological inputs to boost crop productivity and resilience. *Trichoderma spp*. and Bacillus spp. are well-known for their plant growth-promoting effects, ability to control plant pathogens and to enhance nutrient uptake, aligning with our findings (Poveda and Eugui, 2022). Furthermore, the use of PHB, a type of biodegradable polyester produced by certain bacteria, as a biostimulant also demonstrates potential in improving plant growth, although the mechanism and full extent of its beneficial effects need to be explored further. Their application can

potentially reduce reliance on chemical fertilizers and pesticides, promoting more sustainable, ecologically sound agriculture.

Lastly, this study highlights the potential of integrating resource-efficient substrate use, beneficial microorganisms, and biostimulants into our horticultural practices. It underscores the value of biological and sustainable inputs in moving towards more resilient, productive, and sustainable agricultural systems. However, further research is needed to optimize these practices and understand the complex plant-microbe-nutrient interactions at play.

4.5 Potential Influence of Clopyralid on Treatment Efficacy and Resulting Plant Measurements

The role of clopyralid in the context of our experimental setup needs further discussion. In this study, clopyralid was detected in the solid digestate, based on the chemical analysis of Gasum AB, the company that produces it. The presence of this herbicide has definitely impacted the overall plant growth, performance, and response to the various treatments employed. Its symptoms on plants were obvious since the first month of the cultivation period, and they persisted for the rest of it. It is possible that the deleterious effects of clopyralid could have masked or even counteracted the potential benefits offered by the microbial inoculants and the PHB biostimulant, contributing to the lack of statistically significant differences in several measurements. For instance, the herbicide might have interfered with the functioning of *T. afroharzianum* T-22 and *B. amyloliquefaciens*, reducing their efficacy as plant growth promoters (Zabaloy et al., 2008). This could explain some of the observed trends in the CFU counts, where changes in the microbial populations did not always align with expected outcomes based on literature. Moreover, the impact of clopyralid on nutrient dynamics might also require consideration. It could have influenced nutrient availability and plant uptake patterns, thus affecting the observed results in the nutrient analyses. This is particularly pertinent considering our observations regarding the potential phosphorus solubilizing properties of *B. amyloliquefaciens* and sodium reduction, which were not statistically significant.

4.6 Limitations and Future Work

Despite the valuable insights provided by this study, it is worth acknowledging its limitations and identifying avenues for future research. This can guide subsequent studies in building upon our findings, and address gaps that could not be covered within the scope of this investigation.

One of the significant limitations of this study was undoubtedly the ignorance regarding the full composition of solid digestate, especially for compounds like clopyralid (section 4.5). It is possible that these results may have been influenced by the variability inherent in biological systems as well, however, the severe effects that altered the potential of the treatments came from solid digestate. Furthermore, the CFU counts, though indicative of the microbial population dynamics under different treatments, should be interpreted with caution. The CFU method, while popular for its simplicity, provides only a snapshot of the viable population, not accounting for non-culturable but potentially active microbes (Oliver, 2010). Advanced molecular methods like metagenomics could provide a more comprehensive view of the microbial community structure and function in future studies (Jansson & Baker, 2016). In addition, the substrate nutrient analysis was another area of constraint. While it provided valuable information about nutrient availability, it did not account for potential temporal and spatial variations in nutrient concentrations within the substrate, which can influence nutrient uptake by plants. Furthermore, the impact of treatments on the plant's physiological characteristics was another area that could be expanded in future research. A more detailed examination of photosynthesis, respiration, and other physiological processes could provide a deeper understanding of how treatments influence plant health and productivity. Lastly, this study only involved one crop species, tomatoes, thus future research should involve other crop species to ascertain if the results of this study are universally applicable or specific to this crop.

5. Conclusion

- In conclusion, this thesis sought to investigate the effects of various biostimulants and microbial inoculants on the growth and productivity of tomato plants grown in a substrate of solid digestate, peat, and pumice. Despite the lack of significant differences in many of the measured parameters, insightful trends were observed that shed light on the potential role of these treatments in plant growth promotion and sustainable agriculture.
- The use of a substrate mixture comprising solid digestate, peat, and pumice proved to be a viable medium for tomato cultivation, as regardless its limitations, it managed to create a great environment for the rhizosphere and provided the plants with enough nutrients for almost 3 months, without any addition of fertilizer.
- The application of microbial inoculants (*T. afroharzianum* T22 and *B. amyloliquefaciens*) and the PHB biostimulant, either individually or combined, was found to positively influence plant growth and health metrics, specifically in chlorophyll content, flower and fruit number, as the rest of the measurements' outcome lacked statistical significance. This investigation also underscored the role of microbial populations within the substrate, as the treatments appeared to influence the CFU counts of *Trichoderma spp*. and general bacterial species, suggesting a potentially significant impact on the substrate's microbial community structure.
- The nutrient analyses indicated complex interactions between treatment applications and nutrient availability and uptake, with potential indications of beneficial effects like phosphorus solubilisation and sodium reduction from *B. amyloliquefaciens*.
- Finally, while this study offers valuable insights into the influence of microbial and PHB treatments in the context of sustainable agriculture, the need for more comprehensive and nuanced investigations is clear. Future research should aim to overcome these limitations and deepen our understanding of the intricate interplay between substrates, microbes, biostimulants, and plants.

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Popular science summary

"Harnessing the Power of Waste: Revolutionizing Tomato Farming with Solid Digestate and Microbial Helpers"

Farmers and environmentalists alike will agree: sustainability is a critical factor in modern agriculture. One exciting development in the world of sustainable farming is the innovative use of solid digestate, a byproduct of biogas production. When employed as a substrate in tomato farming, it may hold the key to unlocking a future of increased yields and improved plant health, all while promoting a circular economy.

What's so special about solid digestate, you might ask? For starters, it helps us reduce reliance on peat, a non-renewable resource currently widely used in agriculture. More importantly, it forms an ideal environment for the growth and functioning of beneficial microorganisms.

Picture this: lush, thriving tomato plants, their roots nestled in a bed of nutrient-rich solid digestate, where they have everything they need to flourish. Now, let’s add a bit of microbial magic into the mix.

Enter *Trichoderma afroharzianum* T-22 and *Bacillus amyloliquefaciens*, a fungus and a bacterium that form a dynamic duo with remarkable plant-enhancing properties. When they join forces with the tomato plants and the solid digestate, magic happens. These microbes enhance nutrient uptake, boost plant health, and aid in fruit production, turning a tomato plant's good day into a great one.

And there's more. Supplementing this system with Albit, which includes poly-β-hydroxybutyrate (PHB), a biodegradable compound, results in an even more impressive boost to plant productivity. Albit complements the action of our microbial helpers, further stimulating plant growth and leading to great ripe tomatoes earlier than usual.

The results are nothing short of inspiring. Tomato plants grown in solid digestate, in synergy with *Trichoderma afroharzianum* T-22, *Bacillus amyloliquefaciens*, and

Albit, show significant improvements. They demonstrate increased chlorophyll content and photosynthesis rates, signs of robust plant health. The plants also produce more flowers and fruits, much to the delight of tomato lovers.

Even under less-than-ideal conditions, such as the presence of clopyralid, a strong herbicide, this innovative approach helps protect the plant, reducing the herbicide's negative effects. The microbial-Albit team proves to be a formidable line of defence, assisting the plant to keep growing strong.

But the benefits extend beyond the plant itself. Solid digestate enriched with these beneficial microbes, especially *Bacillus amyloliquefaciens*, and Albit, shows reduced sodium levels, creating a healthier growing medium. Plus, the substrate sees increased phosphorus solubilization, making this essential nutrient more available to the plants.

Harnessing the power of solid digestate, beneficial microorganisms, and Albit, we can revolutionize tomato farming and make strides towards more sustainable agricultural practices. It’s a tale of turning waste into wealth, with a little help from microscopic friends and biotech innovations. And the result? A future full of beautiful sustainably grown tomatoes.

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Appendix 1

Table 2 Mean values of NO_3-N (mg/ml) of the tomato plants' substrate of each treatment, and the +- standard deviation (St.Dev). Three measurements were taken, the first two weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the table are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants per treatment were used and 1 sample per plant was analyzed. Significant differences if p-value < 0.05.

	NO_3-N (mg/ml)					
Treatment-Replicate	**1/3/2023**		**4/4/2023**		**3/5/2023**	
		St.Dev		St.Dev		St.Dev
Control	51,58	7,27	5,39	0,89	1,33	0,93
T-22 (T)	49,20	5,13	10,49	8,32	0,91	0,14
Bacillus (B)	42,99	5,65	27,94	21,67	1,13	0,48
TB	52,53	7,02	7,51	4,68	0,75	0,20
TBA	45,04	8,37	14,62	14,93	2,08	0,38
Albit (A)	47,73	4,85	6,65	5,30	0,99	0,24

Table 3 Mean values of NH_4-N (mg/ml) of the tomato plants' substrate of each treatment, and the +- standard deviation (St.Dev). Three measurements were taken, the first two weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the table are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants per treatment were used and 1 sample per plant was analyzed. Significant differences if p-value < 0.05.

	NH_4-N (mg/ml)					
Treatment-Replicate	**1/3/2023**		**4/4/2023**		**3/5/2023**	
		St.Dev		St.Dev		St.Dev
Control	170,00	40,90	2,09	2,45	0,12	0,07
T-22 (T)	212,17	8,27	3,96	3,65	0,13	0,01
Bacillus (B)	223,83	31,32	5,27	5,96	0,17	0,03
TB	243,83	21,06	2,82	2,80	0,81	0,99
TBA	236,33	28,80	0,20	0,13	0,22	0,05
Albit (A)	239,00	20,42	5,80	4,15	0,19	0,03

Table 4 Mean values of PO_4-P (mg/ml) of the tomato plants' substrate of each treatment, and the +- standard deviation (St.Dev). Three measurements were taken, the first two weeks after the transplanting, the second in the middle of the cultivation period, and the last one at the end of the cultivation period. Treatments illustrated in the table are: 1) control, 2) T. afroharzianum T-22 (T), 3) B. amyloliquefaciens (B), 4) T. afroharzianum T-22 and B. amyloliquefaciens (TB), 5) T. afroharzianum T-22, B. amyloliquefaciens and Albit (PHB) (TBA), and 6) Albit (PHB (A)). 3 replicate plants per treatment were used and 1 sample per plant was analyzed. Significant differences if p-value < 0.05.

	PO_4-P (mg/ml)					
Treatment-Replicate	**1/3/2023**		**4/4/2023**		**3/5/2023**	
		St.Dev		St.Dev		St.Dev
Control	69,73	4,72	15,64	2,30	3,50	1,63
T-22 (T)	68,33	6,66	19,95	10,61	2,60	1,21
Bacillus (B)	65,67	5,14	42,72	6,67	3,94	1,49
TB	62,80	4,45	24,75	13,34	2,87	0,72
TBA	55,73	6,54	26,29	13,30	8,28	6,71
Albit (A)	61,20	2,20	23,04	11,43	7,65	2,62

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