CZECH UNIVERSITY OF LIFE SCIENCES, PRAGUE

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THE EFFECT OF FERTILIZATION AND BIOSTIMULANTS APPLICATION ON TOMATO PRODUCTION.

DIPLOMA THESIS

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DIPLOMA THESIS TOPIC

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Thesis title:	**The effect of fertilization and biostimulants application on tomato production**
Objectives of thesis:	Objectives The aim of this work is to evaluate different fertilizers and microorganisms (in soil or substrate) on the release of different nutrients in bioavailable form and therefore, improve tomato plants growth ant fruit yield. Hypothesis Applied microorganisms improve the release of nutrients from fertilizers as well as soil pool. This will lead to increase of bioavailable nutrient content in soil and to higher fruit yield.
Methodology:	Master thesis will be based on the evaluation of two experiments: First is the experiment realized in the vegetation chamber of the greenhouses at Czech University of Life Sciences in Prague (year 2015). Tomatoes were grown in nutrient poor substrate. In comparison with substrate only (control - treatment 1), the treatments with different microorganisms were tested. Microorganisms applied in soil were following: Treatment 2: Trichoderma harzianum, strain T22 (Trianum) Treatment 3: Bacillus amyloliquefaciens, strain FZB42 (RhizoVital) Treatment 4: Pseudomonas sp. Strain DSMZ 13134 (Proradix)

Treatment 5: Combination of Trichoderma harzianum, Bacillus subtilis, zinc and manganese (CombiFect A)
Tomato plants were grown until the maturity. The yield of above ground biomass (without fruits) and fruits was evaluated as well as the content of bioavailable nutrients in soil and total nutrient content in mature fruits.

Second experiment was realized in the greenhouses of the Swedish Agricultural University (Uppsala) in the year 2018.
The tomatoes were grown in substrate with different fertilizing or inoculation systems. The evaluated treatments were following:
Treatment 1: Control without application
Treatment 2: Pseudomonas, strain MS100 (further MS100)
Treatment 3: Silage (based on the expected N requirement)
Treatment 4: Silage (based on 1/2 of expected N requirement) (further 1/2N)
Treatment 5: MS100 and 1/2N
During this experiment, plant height and stem length and thickness, total number of leaves, fruits and clusters and SPAD index.

The proposed extent of the thesis:	approx. 50 standard pages
Keywords:	soil microorganisms; plant nutrition; tomatoes; fruit yield

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DECLARATION

STUDENT'S DECLARATION

I KADIR ABUBAKAR SADIQUE, hereby declare that except for references to other people's work which have been duly cited and acknowledged, this action research is the result of my own effort and that it has neither in whole nor in part been presented elsewhere.

SIGNATURE……. DATE…….

(KADIR ABUBAKAR SADIQUE)

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ABSTRAKT

Rostoucí intenzita použití minerálních hnojiv má negativní dopad na biochemické vlastnosti půdy, podílí se na znečištění prostředí a na zdravotních komplikacích populace. Mnoho vědeckých studií naznačuje, že využití biologických substancí a mikroorganismů (tzv. biostimulantů) má potenciál napomoci k redukci vstupů chemikálií do životního prostředí a zároveň podpořit produkci kvalitních potravin. Některé biologické látky, jako např. výtažky z řas, huminové látky, *Trichoderma, Mycorrhizal fungi (Arbuscular mycorrhizal fungi). Pseudomonas sp.* a *Bacillus amyloliquefaciens* se již osvědčiliy jako tzv. biohnojiva zlepšující přístupnost makro- i mikroprvků v půdě, podporující růst kořenů a podílející se na zvýšení kvality produkce. V této dilpomové práci byl sledován vliv mikroorganismů *Pseudomonas, Trichoderma, Bacillus amyloliquefaciens* a směsi biostimulantů v různých režimech výživy rostlin (s nebo bez přidaných organických materiálů) na vegetativní a generativní růst rostlin rajčat a přístupnost živin. Přidání roztoku s bakteriemi nevedlo k pozitivnímu efektu na vegetativní ani generativní růst rostlin rajčat, organické hnojení však u některých variant podpořilo generativní růst. Bakterie dále měly neprůkazný vliv na mobilizaci živin. Důvodem je pravděpodobně fakt, že použitý kmen *Pseudomonas* MS100 byl potvrzen jako produkt podílející se na ochraně rostlin proti patogenům, ale ne z hlediska mobilizace živin. Z dalších výsledků je zřejmé, že bakterie *Pseudomonas*, kmen DSMZ 13134 mobilizovala mangan v půdě. Směs mikroorganismů CombiFect A pozitivně ovlivnila obsah síry v plodech rajčat. Na druhou stranu *Trichoderma* negativně ovlivnila obsah zinku a železa, a stejně odběr železa plody rajčat. CombiFect A měl rovněž negativní vliv na odběr dusíku plody rajčat. Z dosažených výsledků nelze jednoznačně potvrdit pozitivní působení zkoumaných biostimulantů. Z toho důvodu je nutný další podrobnější výzkum.

Klíčová slova: Biostimulanty; bakterie; houby; živiny; růst a generativní vývoj; produkce potravin

ABSTRACT

The use of mineral fertilizers has a negative impact on soil biochemical properties, environment as well as it causes some health implication. Scientists have established that some biological substances and microorganism (biostimulants), can help in reducing the amount of chemical use in food production and improves food quality. Some biological substances such us seaweed, humic substances, *Trichoderma, Mycorrhizal fungi (Arbuscular mycorrhizal fungi). Pseudomonas sp* and *Bacillus amyloliquefaciens* have proven to be biofertilizers, which increase the availability of both macro and micronutrient in the soil and improves root and shoot length of plants as well as increase the yield quality. We examined the effect of *Pseudomonas, Trichoderma, Bacillus amyloliquefaciens* and mixture of the biostimulants in different fertilization regimes, with and without organic materials to analyze their effect on vegetative and generative growth and nutrient availability in the tomato plants. Adding bacteria solution resulted in no positive effect on vegetative growth and generative growth of the tomato plants but adding the organic fertilization resulted some of treatment have better generative growth. Furthermore, in terms of nutrient mobilization it has less impact. The cause for this could be that the strain of *Pseudomonas*, strain MS100 which just improve the growth and crop protection and did not improve nutrient mobilization. By the results, *Pseudomonas*, strain DSMZ 13134 mobilized the Mn in soil. Furthermore, CombiFect A had positive effect on sulfur content in tomato fruit. On the other hand, application of *Trichoderma* negatively influenced the content of zinc and iron in tomato fruits as well as iron uptake. CombiFect A had also negative influence on nitrogen uptake with tomato fruits. Our results are insufficient to definitively state that tested bioefstimulants have positive influence on investigated parameters. Therefore, further research is needed.

Keywords: Biostimulant; bacteria; fungi; nutrient; vegetative growth and generative development; food production

Table of Contents

Table of Contents 1
CHAPTER 1 1
1. Introduction 1
CHAPTER 2 2
2. AIMS AND OBJECTIVES OF THE STUDY 2
Aim 2
CHAPTER 3 3
3. LITERATURE REVIEW 3
3.1. Tomato production 3
3.2.1. What do we know about Biostimulant 4
3.2.2. Composition of biostimulant 5
3.2.3. How do biostimulants work? 6
3.3. Humic substances 7
3.3.1 Nutrient and plant defense. 8
3.4. Seaweed 9
3.5. Trichoderma 10
3.5.1 Nutrient Mobilization / growth promotion 11
3.6. Mycorrhizal fungi (Arbuscular mycorrhizal fungi). 13
3.7. Bacteria as a biostimulant 15
3.7.1. What is Plant growth promoting bacteria / plant growth promoting rhizobacteria (PGPB/PGPR) 15
3.8. What do we know about Pseudomonas sp? 16
3.9. *Pseudomonas sp.* and tomato (*Lycopersicon esculentum L*) 16
CHAPTER 4 18
4. MATERIALS AND METHODS 18
4.1. Experimental site and climate 18
4.2. Experimental design 18
4.3. Plants 20
4.4. Fertilization regime 21
4.5. Growth analysis 22
4.6. B= Czech university of Life sciences (CULS/ ČZU) greenhouse 22
4.7. Analytical methods and vegetation tests 24
4.7.1. Vegetation tests 24
4.7.2. Determining of substrate pH value 25

4.7.3. Determining of nutrients content using CAT extraction procedure 25
4.7.4. Determining of bioavailable calcium content in water extracts 25
4.7.5. Analysis of tomato fruits 25
4.7.6. Determining of total nitrogen content in tomato fruits 26
4.8. Statistical analysis 26
CHAPTER 5 27
5. RESULTS 27
5.1. Vegetative growth 27
5.1.2. Stem thickness 27
5.1.3. Stem length 28
5.1.4. Plant Height 28
5.1.5. Number of leaves 29
5.2. SPAD index of the leaves 30
5.3. Generative growth 31
5.3.1. Number of clusters. Flower development. 31
5.3.2. Number of fruits 32
5.4. Results of tomato experiment in CULS. 33
CHAPTER 6. 38
6. Discussion 38
6.1. Vegetative growth 38
6.1.2 Stem thickness 39
6.2. Generative growth 40
6.3. Discussion of the second experiment at CULS 42
6.3.1. Macronutrients assessment. 43
6.3.2. Micronutrients assessment. 44
CHAPTER 7 46
Conclusion 46
REFERENCES 47

CHAPTER 1

1. Introduction

The current world population is approximately 7.6 billion and been anticipated to reach 8 billion in the coming year 2020. This huge increase coupled with the deteriorating nature of our environment without sufficient innovation to have sustainable food production. The results can be chaos if not an avalanche and humanity can be said to be successful in feeding itself if we are able to provide for our basic nutritional requirement and also our ability to ensure a sustainable food production.

Agriculture has a tremendous impact on global scale in terms of food production to address the two criteria stated above. It has created employment both on the field and the supply chain. However, the World Health Organization has reported that 40 percent of death recorded globally are the result of water, soil and air pollution where agriculture is a one of the contributors. Until now, most research works were mainly focusing on increasing crop yield and quality through the use of chemicals and hormones. But the recent increase in health concerns around the world amongst people on the need to focus on food quality is gaining grounds day by day. People are demanding for chemical free production and to minimize the impact of agriculture on climate change as well as pollution in general.

What then is the way forward?

The way forward is to look at both quality of food produce and the quantity through the use of biostimulants which will reduce a tremendous impact of agriculture on the environment. The use of biostimulant as a means of activating plant physiological responds and secondary metabolites helps in enhancing plant nutrient use efficiency and stimulating plant growth and development which leads to a reduction in fertilizer and chemicals usage. This is the emerging trend to balance both environmental quality, quality of food and a sustainable food production.

Boistimulants contain substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/ benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. They can be of bacteria, plant, seaweed, fungi, “humate-containing” or animal. They have the potential to influence plant micro- and macronutrient uptake, nutrient use efficiency, and withstand biotic and abiotic stress.

CHAPTER 2

2. AIMS AND OBJECTIVES OF THE STUDY

The main objective of this study is to assess the alternative ways of food production from the normal conventional intensification. Through the application of biological material or substances (biostimulants) to enhance plants nutrient uptake and chemical free food production.

Aim

The aim of this work is to evaluate different fertilizers and microorganisms (in soil or substrate) on the release of different nutrients in bioavailable form and therefore, improve tomato plants growth and fruit yield.

Hypothesis

Applied microorganisms improve the release of nutrients from fertilizers as well as soil pool. This will lead to increase of bioavailable nutrient content in soil and to higher fruit yield.

CHAPTER 3

3. LITERATURE REVIEW

3.1. Tomato production

Tomato (*Lycopersicon esculentum L.*) is one of the most common vegetable crops grown in the world and were mostly grown in the field (Olson and Simonne 2004; Chin et al.2011). It is widely consumed globally partly due to its high nutrient content in vitamin C and its antioxidants function (Toor et al.,2006; Erba et al.,2013; Wang et al.,2015; Du et al., 2017; Du et al.,2018). Farmers rely on intense use of mineral fertilizers in the production process, as a way of achieving a better yield. The use of these mineral fertilizers has negative impact on soil biochemical properties (Perrott et al. 1992; Steinshamn et al. 2004; Adesemoye and Kloepper 2009). Nitrogen (N) is essential to plants growth, it is a major determiner of crop yield and quality. A proper application of N fertilizer during the cropping cycle helps in maximizing yield and economic benefits. However, inappropriate use of N fertilizer affects soil quality through nitrate leaching, pollution of groundwater and may also shift the balance between vegetative and reproductive plant growth. Plants may take an excessive vegetative growth than reproductive development rendering losses to the farmer (Du et. al 2018). Despite the negative impact N fertilizers have on the environment, fertilizer use is expected to increase worldwide because of the need to feed the growing population and their nutritional need (Adesemoye and Kloepper 2009). Tomato production on the global scale have increased from 27.6 million tons in 1960 to 177 million tons in 2016, due its nutritional demand and the development of processing industries (FAO 2016; Du et al.,2018).

Tomato plant is stress intolerant plant, it is sensitive to both biotic and abiotic stress, especially temperature and drought (Kaloo, 1993; Petrozza et al.,2014). As a result of the sensitivity, they are mostly produced in greenhouses these days because it is easier to control the growing condition for an optimum yield. They have huge water and fertilizer demand due to its long growing period and temperature requirement (Du et al.,2018).

Why the need to decrease the amount of fertilizer usage in food production.

As a result of the growth in population and demand for food, there is a need for a paradigm shift in food production process to limit the amount of fertilizer use in other to save the environment (Adesemoye and Kloepper 2009). Until now, most research works were tilted towards increasing crop yield and quality through the use of chemicals and hormones. But the recent increase in health

concerns around the globe among the people on food quality is gaining grounds day by day. People are demanding for chemical free production and to minimize the impact of agriculture on climate change as well as pollution in general (Mahmood, 2017).

Research conducted by scientists of Cornell University captured in the World Health Organization report indicates that 40 percent of the death reported globally are as a result of water, soil and air pollution. It indicates that environmental pollution coupled with the growth in world population is a major cause of increase in disease prevalence in our societies. The pollution increases in our societies' makes human susceptibility to infections (Pimentel, 2007). However, with the current world population of 7.6 billion, and it is projected to increase to approximately 8 billion by 2020 (worldometers projections 2018). Which means there is constant pressure on agricultural to produce enough to meet the population demand in other to avoid famine. To meet these global demands and reduce pollution, there is a need to look into alternative way of producing food in a sustainable manner (Glick, 2012). The alternative way proposed by scientists is to use biological substances which includes microorganisms. These microbes help in improving plant growth and development as well as its ability to withstand abiotic stress (Colla and Rouphael, 2015).

3.2.1. What do we know about Biostimulant

The initial application of biostimulants were not promising because many of the microbes were produced with less scientific base, but of commercial interest to the producers. Quality control and production was geared towards marketing purpose (Colla and Rouphael, 2015). However, the recent understanding of biological processes in plant and microbe interaction, has brought about a significant improvement in biostimulants. Which makes its application beneficial to plants (Colla and Rouphel, 2015). Biostimulants differ from chemical fertilizers because they act on plant physiology and metabolism to enhance soil structure with less nutrient concentration. They are capable of improving plant root growth and development (Berlyn & Russo 1990; Nardi et al. 2006; Petrozza et al. 2013a, 2013b; Bulgari et al.2015).

Biostimulant can be defined as substances of "biological origin" that can either, be of bacteria, plant, seaweed, fungi, "humate-containing" or animal. That has the potential to influence plant

nutrient uptake, nutrient use efficiency, and withstand biotic and abiotic stress (Calvo et al.,2014; Yakhin et al., 2016; Ugena et al., 2018)

According to the European Biostimulant Industry Council (EBIC) "Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/ benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides" (European Biostimulants Industry 2012a; Calvo et al.,2014). The north Americans made a clear distinction of biostimulants and nutrient. They defined biostimulants as "substances, including microorganisms, that are applied to plant, seed, soil or other growing media that may enhance the plant's ability to assimilate applied nutrients, or provide benefits to plant development. Biostimulants are not plant nutrients and therefore may not make any nutrient claims or guarantees" (Biostimulant 2013; Calvo et al., 2014).

Biostimulant have been proven to be the best sustainable production option as many scientific publications has suggested (Bulgari et al.2015). They are obtained through "chemical hydrolysis" of proteins and peptides, which can be from plant source, animal waste, bacteria or fungi (Halpern et al.,2015; Koleska et al. 2017). Biostimulant can be applied to plants either on the soil by root inoculation or by leaf. The method of application depends on what constitute/ composition of the biostimulant and the results you need (Kunicki et al. 2010; Bulgari et al.2015). They can act or stimulate growth and development only if they are able to penetrate the plant tissue. However, the success of it and rate of work depends on its permeability, weather conditions and other factors (Kolomaznik et al. 2012; Pecha et al. 2012; Bulgari et al.2015). Most biostimulants acts as elicitors, they increase plant fitness against pathogens through systemic acquired resistance. Others biostimulants helps in microelements and nutrient mobilization for plant uptake. (Adani et al., 1998; Vernieri et al., 2006; Tuteja, 2007; Paradikovic et al.,2011).

3.2.2. Composition of biostimulant

Biostimulant comprises of several materials that are capable of stimulating the soil biota and to enhance plant growth (Hamza and Suggars, 2001). These materials or substances are derived from "biological or natural" sources (Biostimulant 2013; Calvo et al., 2014). The EBIC and the North American consortia categorized biostimulant into Microbial inoculants, Humic acids and

fulvic acid, protein hydrolysates (amino acids) and seaweed extracts (Calvo et al., 2014). Microbial inoculants include bacteria (*Pseudomonas*) which is a free-living bacteria and fungi and arbuscular *mycorrhizal* fungi (AMF) (Berg 2009; Dodd and Ruiz-Lozano 2012; Vessey 2003; Calvo et al., 2014). Fungi such *Trichoderma* and arbuscular mycorrhizal fungi are able to stimulate plant growth and development. Trichoderma is capable of producing enzymes and antibodies (Holečková et al. 2017). Whereas Arbuscular mycorrhizal fungi are found to contain five "phosphate transporter" genes in its fungi hyphae (Sokolski et al., 2011; Wang, 2017). According to Hamza and Suggars, (2001), these substances are have reported to providing the following benefits (Table 1).

Table 1: Summary of the biostimulants benefits (Hamza and Suggars, 2001)

Stimulate plant responses and work in all weather conditions	Result in better performance
Increase profits, cut operating costs, lead to 50% reduction in fertilizer	Produce deeper roots
Increase natural plant toxins, repelling pests	Improve stress tolerance
Increase microbial root protection from soil pathogens.	Accelerate establishment
Increase soil nutrient reserve up to 3000%	Increases Cation Exchange Capacity
Improve root development	Enhances fertilization and reduces leaching
Build yields	Detoxify chemical residues and heavy metals
Improve taste and shelf-life	Make urea a long-life nitrogen
Improve drought tolerance	Improve seed germination rates
Increases nutrient uptake	Increase stomatal opening and plant
Stimulate plants' immune system	
Produce better color	

3.2.3. How do biostimulants work?

The processes involved in biostimulant activities in plant can be analysed through molecular biology technologies. That is, either "micorray or transcriptome" which helps to reveal the actions of biostimulant in the pathway (Santaniello et al. 2013; Bulgari et al.2015). There has been an extensive work done on plant hormones and their role in plant growth and development. The phytohormones regulate several plant tissues-related activities, ranging from "stem elongation, root initiation and tissue differentiation", plant defence and stress tolerance (Hamza and Suggars 2001). The phytohormones are categorized into five groups and each has a role in plant growth and development. They are for example gibberellic acid, auxins, cytokinin's, abscisic acids, and ethylene. Gibberellic acid stimulates shoot elongation (making the plant to grow tall), seed germination, ripping of fruits etc. Abscisic acid regulates seed dormancy and stomatal conductance in plant defence against pathogen (Hamza and Suggars 2001). Auxin regulates most aspect of plant growth and development (Grossman 2010; Duca et al., 2014). The most common auxin is indole -3-acetic acid (IAA), which is produced by bacteria and fungi. They are responsible for cell division, root sprouting, leaves and flower development (Philips et al. 2011; Duca et al., 2014). Cytokinin helps the plants in nutrient mobilization (Hamza and Suggars 2001). There have been several studies that indicates how plants treated with biostimulant has increase chlorophyll content in plants and "net photosynthesis" for that matter (Ferrini and Nicese 2002; Amanda et al. 2009; Ertani and Nardi 2013; Bulgari et al.2015).

3.3. Humic substances

Humic substances are products of decomposed organic materials from peat, dumping sites, lignite's, (Sharif et al., 2002; Bulgari et al., 2015) and droppings from animals, as well as "microbial residual" (du Jardin 2015). These substances are able to facilitate plant nutrient uptake and enhances growth and development. The growth and development response are not only connected to the nutrient content in the Humic substances but the microbial activities as well as the interaction between the "transport membrane" and the growth regulate in the plant system (Canellas et al., 2015). Several scientific publications have indicated that "humic substances" are very diverse and non-homogeneous in their molecular structure (Schnitzer & Khan 1972; Aquino et al., 2011; Klucakova and Veznivkova 2017). Their composition can vary depending on the following: i) geographical location, ii) how long the deposit has been there, ii) the climate and the biological condition (Thom et al., 1989; de Melo et al., 2016).

Humic substances comprise of two main parts, that is; Fulvic acid and Humic acid. The two are distinguished based on their "solubility at pH level". Humic acid is the "precipitated" part and the fulvic acid is the part that is left in the solution. The fulvic acid usually represent the chunk of humic substances with humic acid been the smallest part (Andrews & Huck, 1996; Langlais et al., 1991; Rodriguez & Nunez 2011; Rodriguez et al., 2014).

Their effect on plant is related to how they are able to change the roots structure, and the micro activities in the rhizosphere, which changes the soil chemistry to facilitate the plants nutrient uptake (Lucas, 2001; Canellas et al., 2015). They are generally known for their increasing solubility from neutrality to alkalinity state of substance (Stevenson, 1994; de Melo et al., 2016) and their ability to release nutrient in a small quantity to plants as well as mitigating the pH and alkalinity of the soil. The soil texture is enhanced, which increase its ability to hold water to ensure the plants gets the needed water it deserves (Stevenson, 1994; Giannouli et al., 2009; Doskočil et al., 2018). They are reported to have caused plasma membrane activation through the binding of the ATPases. This then cause the maize root cell walls to loosen up and increase in the root length "root elongation" (Jindo et al., 2012; du Jardin 2015) and enhances leaf nutrient absorption as well as chlorophyll biosynthesis in the plant (Bulgari et al., 2015).

3.3.1 Nutrient and plant defense.

Humic substances increase plants respiratory rate and increase in enzyme activities to help in amino acids conversion by providing them with carbon. However, it is not known yet whether humic acid is able to interfere or play a role in the plant signaling pathway such as elicitor and receptor activities (du Jardin, 2012; du Jardin, 2015). The common proposal put forward by scientists in terms of plant defense is humic acid ability to stimulate "Phenylpropanoid metabolism", which helps plant against both biotic stress through the production the production of secondary metabolites (coumarins, lignans and flavonoids). This was shown in the maize on how increase in enzyme activities, facilitated by the humic substance increased its ability to deal with stress through modulation (Olivareset al., 2015; Schiavon et al., 2010; du Jardin, 2015). It is estimated that, there are over 240 auxin-like molecules described in the scientific literature and humic substances is said to contains some of these auxin-like molecules. These molecules play some roles both within and outside the host cells. They cause the variation in the host root system by proton activation, which is triggered by the humic substances (Canellas et al., 2015).

In terms of direct nutrient acquisition, the increased in enzyme activities regulate plant nutrient uptake and assimilation. They increase the enzyme activities control in the citric acid cycle (TCA cycle), which causes the exchanges in carbon and nutrient metabolism (Colla et al., 2014; du Jardin 2015). There are several reports on how humic substances have stimulated growth in several plant species including pepper, maize, soybean, rice, wheat, tomatoes and cucumber. It helped in enhancing root development in these plants and also growth in the roots of seedling. However, most of the experiment were carried in a controlled environment (Adani et al., 1998; Canellas et al., 2011; Calvo et al., 2014).

3.4. Seaweed

As explained on the composition of biostimulant by Calvo et al. (2014) in Ugena et al. (2018), which includes seaweeds. Seaweeds as a biostimulant is well studied in terms of its ability to stimulate or enhance plant growth (Du Jardin 2012; Lotze and Hoffman 2016) and against stress through stimulation of "antioxidant activities" (Lolo-Luz et al., 2014; Battacharyya et al., 2015). These species are purely from the marine ecosystem which makes them very common in the coastal areas. Studies have shown that, there are about 9000 of them, and has been broadly categorized in to three based on their colours: *Phaeophyta*, "brown algae", *Rhodophyta* "red algae" and *Chlorophyta* "green algae" (Khan et al. 2009). Seaweed extracts has gain prominence recently in terms of its ability to stimulate growth and development. They are mostly used in liquid form to spray on crops (Crouch & Van Standen 1994; Rathore et al., 2008). Algae extracts have proven to be an efficient biostimulant because with a little amount applied on plant, it can induce several physiological responses and improve flower development as well as fruit quality (Khan et al. 2009; Battacharyya et al., 2015). The extract contains amino acids, abscisic acid, auxins and cytokinins which helps in crop development (Mooney and Van Staden 1986; Rathore et al., 2008).

Seaweed used on plant increases its stress tolerance through the receptor activities in the following pathway: the signaling process of jasmonic acid and salicylic acid as well as increases pathogenesis-related (PR) protein in the plant. Which enhances the plant ability to defend itself against pathogen (Moon and Anderson 2003, 2006; Craigie 2011). There is a report on how cotton seedlings were prone to "*Xanthomonas campestris"* but the seeds soaked in algae extract for a considerable number of hours before sowing proved a strong resistance to the bacteria (Raghavendra et al., 2007; Craigie 2011)

Of all the categories of seaweeds, the brown seaweed is one of the most commonly used in agriculture and has been well studied. Within the brown seaweeds, *Ascophyllum nodosum*, *Laminaria spp.*, *Fuccus spp.*, *Sargassum spp.*, and *Turbinaria spp.* are used as biostimulant in food production (Khan et al.,2009). Scientific studies have shown that brown algae contain some macro nutrients that are vital for plant growth and development. They contain high amount of calcium, magnesium, potassium, phosphorous and sulphur (Verkleij 1992; Sharma et al. 2014; Lotze and Hoffman 2016). There have been reported how an extract from brown seaweed was used on tomatoes which then facilitate the tomatoes uptake of manganese (Mn), zinc as well as its enhanced its chlorophyll level. The extract also made an impact on the germination period, and its fruits weight and quality (Eyras et al., 2008; Dobromilska et al.,2008; Khan et al., 2009; Battacharyya et al., 2015).

Rathore et al., (2008), reported that an application of red algae extracts on soybean showed an increase in straws yield and grain yield of the soybean by 15% higher than the control (without extract). There was also an increase in the soybean plant in its nutrient (nitrogen, potassium, phosphoruos and sulphur) uptake by 12.5%, 15% and 10% respectively than the control (Rathore et al., 2008)

3.5. Trichoderma

Trichoderma spp. are avirulent symbiotic species that means they do not cause harm to their host and are filamentous in nature. Mostly, they are used as biopesticides, as nutrient mobilizers and as growth promoters due to their ability to alter plant root structure (Harman et al., 2004; Harman 2006; Molla et al., 2012). Trichoderma spp. are widely known for their ability to suppress plant pathogen and kill them (Benitez *et al*., 2004; Verma *et al*.,2007; Tucci et al., 2011).

They are capable of stimulating plant growth as well as capable increasing plants defence against stress through the production of antimicrobial compounds (Handelsman & Stabb 1996; Colla et al. 2015). These species of fungi are very common in many parts of the world (Chaverri & Samuels, 2003; Akladious and Abass, 2014). They do not have specific environmental preference (Harman et al., 2004a; Akladious & Abass, 2012). However, their relationship with their host or plant is mutualistic, that is exchange of root exudates and facilitating plant root growth. These species have been widely studied on their potency to promote plant growth and induce plant resistance by competing with some deleterious microbes in the rhizosphere as well

as production of antibiotics (Adams et al., 2007; Bais et al., 2006; Akladious and Abass, 2012). Trichoderma species have been found to stimulate plant micronutrient mobilization like Zn, Cu, Fe and Mn and production of secondary metabolites that stimulate plant hormones like indole-3-acetic acid and auxin like (Colla et al. 2015).

3.5.1 Nutrient Mobilization / growth promotion

Akladious and Abass, 2012, reported an increase in nutrient mobilization in maize plant treated with Trichoderma spp strains. The mechanism used by the fungi species is by secreting some chemical compounds which enhances the host by altering the root system and create more branching. These branches move deeper and far to access nutrient and water for the host (López-Bucio et al., 2015). So, the use of Trichoderma as a biofertilizer could increase plant nutrient availability (Bal & Altintas 2006; Molla et al., 2012). Trichoderma helps in the reducing the rate of fertilizer use there by reducing chemicals substances in food production as well as pollution of water bodies, since the fertilizer use efficiency of the plant is enhanced (Tucci et al., 2011). However, the success of the *Trichoderma spp*. to its host is not automatic, it depends on the plant species and also some specific genotype. There was an experiment run on some selected strains of *Trichoderma* on lettuce, they yielded a positive result in terms growth promotion (Ousley et al.,1994; López-Bucio et al., 2015). But when same strain was used by Baker (1988) on Radish and pea, the results wasn't promising in terms growth promotion (Tucci et al., 2011; López-Bucio et al., 2015). Some results from some crops and *Trichoderma* spp. Are summarized in (table 2).

Table 2. Overview of the results form experiments with *Trichoderma*

Crop	**Trichoderma species/strain**	**Mode of application**	**Experimental conditions**	**Effects**	**Reference**
Arabidopsis	*Trichoderma virens* and *Trichoderma atroviride*	Seedling were grown in Petri dishes and inoculated with 1 × 106 conidia	In vitro	Increased lateral root formation and biomass production	Contreras-Cornejo et al. (2009)
Bean	*Trichoderma harzianum (ALL 42)*	Seeds were immersed in a spore suspension containing 2.4 × 108 conidia/ml	In vitro	Increased overall plant size and the number of lateral roots	Pereira et al. (2014)
Cherry	*Trichoderma harzianum T-22*	Plants were inoculated with approximately 50,000 conidia	In vitro	Increased shoot growth and root development	Sofo et al. (2010)
Chickpea	*Trichoderma sp.*	Agar plates were inoculated with a fungal mycelial disc of 5 mm diameter	In vitro	Increased solubilization of inorganic phosphate	Rawat and Tewari (2011)

Cucumber	*Trichoderma harzianum*	5 × 106 conidia per g of soil or sprayed on roots at a concentration of 1 × 108 conidia/ml	Greenhouse	Promoted seed germination, vegetative growth and flowering	Chang et al. (1986)
Lettuce	*Trichoderma hamatum strain GD12*	Trichoderma bran inoculum added to soil before sowing	Microcosms	Promoted root and shoot growth	Studholme et al. (2013)
Lettuce	*Trichoderma atroviride MUCL 45632*	The substrate was supplied with prepared tablets containing 4.5 × 105 conid	greenhouse, and field conditions	Enhanced shoot and root dry weight, and chlorophyll content	Colla et al. (2015a)

(López-Bucio et al., 2015)

3.6. Mycorrhizal fungi (Arbuscular mycorrhizal fungi).

Mycorrhizal fungi are soil organism that create an interaction between the soil mass and the plant root system (Alizadeh 2010; Alizadeh 2012). They connect plants below ground through the hyphal in terms of resource movement between coexisting plants. And, the interactions process plays a key role in the cycling of carbon, phosphorus, and nitrogen in the ecosystem. There are about four major types of mycorrhizis, that is arbuscular mycorrhiza (AM), ectomycorrhiza (EM), orchid mycorrhiza and ericoid mycorrhiza. They are categorized based on their structure and interaction process (Heijden et al.2014).

Arbuscular mycorrhiza fungi (AMF) are obligates biotrophs that takes up carbon from the roots of their host and in return improve their host nutrient availability supply mainly in terms of phosphate

ions (Smith & Read, 2008; Karasawa etal., 2012; Zhang et al. 2016). They live in the plant root cortex, either on the surface of the plant root or around the epidermal cells (Heijden et al.2014). When plants detect the presence of AMF then it begins to move the calcium in the root epidermal cells and then activate the plant symbiosis genes (Kosuta et al., 2003; Parniske et al.,2005). It must be noted that the fungi cannot complete its life cycle in the absence of its host (plant) and requires a favorable environmental condition (temperature and humidity) in other to develop fungi hyphae (Parniske et al.,2005). AMF are associated with several plant species on the earth in terms of life process and nutrient acquisition. They need to colonize the roots of their host before they can complete their life cycle (Smith and Read 2008; Jansa and Gryndler ,2010). The relationship between these two-unrelated species is cooperative. The mutual benefit is relative in the sense that is not all plant that benefit from the AMF hyphae. The AMF may select microbes that it wants to cooperate based on how effective the host can be in terms of giving them such metabolites to initiate the interaction process in hyphae production (Zhang et al.2016), which is then followed by enzymes production and release of organic compounds to provide them with their host the needed nutrients for growth and development (Marschner, 1998; Rouphael et al., 2015, Zhang et al.2016). The nutrient transfer process by the fungi hyphae could go beyond 4 cm, from the nearest host plant root but the effectiveness of the element's absorption depends on the number of roots in the soil (Alizadeh 2012). AMF is one of the commonness symbians used in horticulture crops, it is capable of bonding with 80-90% "land plant species" (Newman and Reddell 1987; Rouphael et al., 2015) including *Solanaceae* (tomato, pepper etc), *Alliaceae* (onion, garlic etc), fruit trees and ornamentals (smith and Read, 2008 Rouphael et al., 2015).

The fungi play other roles to their host aside nutrient mobilization, they produce a protein called glomalin which is vital for plant. It is able to store carbon and nitrogen as well as its role in carbon sequestration (Wright et al., 1996; Jamiołkowska et al., 2018). The glomalins produced by the fungi provides a protection for the "mycelium hyphae" which serves as a store or medium for movement water, nutrient to their host. This improves the plants nutrition and hence increase in crop yield Nichols, 2004; Gałązka & Gawryjołek, 2015; Jamiołkowska et al., 2018).

Silva et al 2018, conducted a study on cowpea (*vigna unguiculata*) in a greenhouse on two different type of soil with different chemical characteristics, in other to evaluate grain yield, mycorrhizal root colonization and AMF spore number. The results were positive in terms of phosphorous uptake by the cowpea plant and there was also evidence of mycorrhizal colonization except the

treatment that was not inoculated. This root colonization serves as a mediated biocontrol measure since other micro-organism who may be harmful to the plant by infecting the root system will not get the chance to do that (Schouteden et al.2015).

3.7. Bacteria as a biostimulant

Biostimulant contains microorganisms and other material and bacteria are the most abundant microbes in soil (Schoenborn et al., 2004; Glick 2012). Microbes that are involved in nutrient mobilization or biofertilizers are considered as "biostimulant". Biofertilizers are any biological substances that when applied to plant, has the potentials of mobilizing nutrient as well as increasing plant growth and development (Vessey 2003; Calvo et al., 2014). Bacteria application is of two main reasons, as a biocontrol agent, that's preventions of pathogen infections in their host or as a biofertilizer that is nutrient mobilization (Bashan and Holguin 1998; Calvo et al., 2014). These beneficial ones which are component of biostimulants are called Plant growth promoting bacteria /plant growth promoting rhizobacteria (du Jardin, 2015; Nguyen et al., 2018). They are "free living" bacteria within the rhizosphere and are able to make insoluble nutrient be available to their host (Bashan et al., 2014; Calvo et al., 2014).

3.7.1. What is Plant growth promoting bacteria / plant growth promoting rhizobacteria (PGPB/PGPR)

Since the discovery of plant growth promoting bacteria (PGPB) and its associate symbionts in 1888 by Hellriegel and Wolfarth, it has played a major role in food production around the globe (Bashan et al., 2013). The term rhizobacteria was introduced in 1978 by Kloper and Schroth to the bacteria community which are capable of inducing growth and development as well as reducing the susceptibility of plants to disease (Battacharyya and Jha 2012).

Plant growth promoting bacteria (PGPB) plays a crucial role in nutrient mobilization for its host as well as increasing the fitness level of the host against biotic stresses. It does these through "phytohormones synthesis" in other to make the nutrients that are out of reach of its host be available for use (Van Peer and Schippers, 1989; Lugtenberg et al., 1991; Weller & Thomashow,1994; Domenech et al., 2006). The bacteria are able to convert atmospheric nitrogen to enhance plant growth as well as conversion of both organic and inorganic phosphate to stimulate plant growth (Majeed et al., 2018). They function properly when they are provided with a perfect

microenvironment and given the needed protection till, they are well established (Bashan et al 2013). PGPB are simply the bacteria that promote plant growth, which encompasses those free living in the rhizosphere which forms symbiont with their host (example is *Rhizobia* spp. And *Frankia* spp.). They help their host in nutrient acquisition, modulating the phytohormones and induces host resistance to pathogenic agents (Glick, 1995; Glick 2012). PGPR is defined as bacteria that is connected to the rhizosphere which is capable of stimulating growth and development in its host when they get in contact. Studies conducted by Majeed et al. (2018), with sunflower showed an increase in phosphorus availability to the plant as a result of bacteria (*Pseudomonas*) application. They are easy to establish in the soil biota because of their adaptability to different environmental conditions (Cook 200; Bhattacharyya and Jha 2012). However, the heterogeneity of the soil biota could affect the bacterial activities. When the bacteria are unable to find an empty spot to colonize within the soil and has to compete with the "native microflora who is well adapted to the soil. This can result in the ineffectiveness of the bacteria inoculant (Bashan et al., 2013).

3.8. What do we know about Pseudomonas sp?

The family of Pseudomonas sp. have proven to be biofertilizer-PGPR. One of the species identified is *P. fluorescens*, which has the ability to produce phytohormone cytokinins, in soyabean (Vessey 2003). Cytokinins are involved in cell division, cell enlargement and tissue expansion which affect apical dominance. Some research has shown that the presence of *Pseudomonas* spp. has led to an improvement in soil health and activities of different enzymes. They also found that Pseudomonas increase the root and shoot length and increases crop yield (Nosheena et al., 2018).

3.9. *Pseudomonas sp.* and tomato (*Lycopersicon esculentum L*)

Nassal et al. (2018) conducted an experiment with different Pseudomonas sp. treatments in tomatoes. Using a RU47 strain of Pseudomonas increased the P availability in the soil, which lead to improve plant growth like higher stem diameter, bigger shoot biomass and bigger leaf area in this experiment due to phosphatase activity in the rhizosphere of tomatoes. Another result of using bacteria has the potential to improve the quality of the fruit and will be more flavorful, tasty, and

nutritional through the changes in acid-TSS ratio. This makes the organic tomato product easier to sell (Dudás et al., 2017).

When choosing organic fertilizers in some cases, the type of organic fertilizer use is important too. Rock phosphate (RP) is mostly recommended for organic farming systems, even though is not an organic fertilizer but its direct application can increase the crop yield and improve the phosphorus level within the rhizosphere (Kumari and Phogat, 2008). Using Pseudomonas sp. can reduce the use of mineral fertilizers and make the organic tomato production more effective, but there are still several questions about how we should use this microbe in different cultivars, bacteria mixtures and fertilizers (Bona et al., 2018).

CHAPTER 4

4. MATERIALS AND METHODS

A= Swedish university of agricultural science (SLU) greenhouse

4.1. Experimental site and climate

The tomato plants were grown in a greenhouse at the Swedish University of Agricultural (SLU) located at Ultuna, Uppsala. It was conducted between 22nd of March 2018 to 9th of May 2018. The environment of the greenhouse was regulated. There was a daylight of 16 hours and a night period of 8 hours. Temperature during the day was 20 ºC and during the night 16 ºC. The relative humidity was between 43% and 57% as the minimum and maximum respectively during the period of our study.

4.2. Experimental design

The project was part of a bigger project with nine treatments. In this study only five of the treatments were observed. To the comparison, treatment of organic fertilizing Blomstra produced by Orkla Ltd is mentioned in the chapter results and discussion. But, this treatment was not investigated in detail so is not further described in this chapter.

Table 3. The five treatments included in the project.

A	Control = Unfertilized	No nutrients added to the soil
B	Control = Bacteria solution	Bacteria added to the soil
E	Silage	Expected N requirement added to the soil as slow-release organic fertilizer
F	Silage	Half of the expected N requirement added to the soil as slow-release organic fertilizer
G	Silage +bacteria solution.	Half of the expected N requirement added as slow release organic fertilizer and bacteria solution in the same amount as in control B

The design was randomized with four replicates. The plants were located in the greenhouse following the pattern shown in Figure 1 and 2. There were 28 tomato plants labeled (x) placed around the plants under study which were 36 plants. Out of the 36 plants under study, 20 of them were part our study.

Figure 1. The (x) represents protection plants with the objective to protect the experimental plants for example from the border effect.

x	x	x	x	x	x	x	x
x	1F	1B	1G	2C	2D	2J	x
x	1H	1D	1C	2A	2G	2E	x
x	1J	1A	1E	2H	2F	2B	x
x	3C	3E	3F	4G	4H	4A	x
x	3B	3G	3J	4D	4E	4F	x
x	3D	3H	3A	4J	4B	4C	x
x	x	x	x	x	x	x	x

Figure 2. A picture showing the experiment set up with the treatments labeled on the read coloured wooden markers stick in the boxes. This gives a practical picture to the setting in figure (1). Distribution of the plants.

4.3. Plants

The tomato cultivar used was 72-397 RZ (improved cv. Arvento) which is resistant to diseases and were grown organically. The seeds were sown on the 26th of January 2018 and the grafting took place eleven days later, on the 6th of February 2018, onto Maxifort rootstock which provides the plants extra vigorous growth. The seedlings were grown under controlled conditions before they were transplanted.

Transplanting date was on the 22nd of March 2018. The plants were transplanted to boxes measuring 35 x 56 cm (0.2 m^2). They needed 40 kg of fresh greenhouse soil per box which means a total amount 1440 kg of soil (36 boxes x 40 kg fresh greenhouse soil). The dry matter content of the soil was 64 %, resulting in 25.6 kg of dry soil per box. Experimental area was regularly watered using drip irrigation system and weed control was regularly done.

In order to facilitate pollination process after the flower emergence, bumblebees were introduced in week 15. They were contained in a box with some holes through which bumblebees could go out.

4.4. Fertilization regime

Silage was added in the soil during the soil preparation (1st March 2018) of the experiment. The table below (Table 4) shows the treatments and the amount added in each box.

Table 4. Amount of silage added to treatments E, F and G.

Treatments	Amount of nutrient added (g) per box		
	Fresh weight	Dry matter	N
E	1 910	516	15
F & G	955	258	7.5

Bacteria solution

Bacteria was added as bacteria solution, *Pseudomonas*, strain MS100 (Lantmännen BioAgri AB). Amount added in concentrated form around the plant,

Table 5:

- Occasion 1 = week 10= 15 mL solution + 185 mL tap water per box
- Occasion 2 = week 13 = 20 mL solution + 180 mL deionized water per box.

Table 5. Amount of bacteria solution added to treatments B and G.

Date of addition	Bacteria solution			
	Treatments B, G	Volume weight 1.02	% N of FW	g N
March 6th	15 mL	15 ml = 15.3 g	0.26	0.04
March 28th	20 mL			0.05

4.5. Growth analysis

Weekly data recordings were taken regarding the plant parameters; on vegetative and the generative stages.

At the vegetative stage, we recorded weekly data on the following plant parameters using a ruler and a precision pocket Vernier caliper (150mm, Format, Wuppertal, Germany):

- Stem growth/length measured from the cotyledonary node which is marked weekly with a marker to the shoot tip. This is done with a foldable ruler every week to see the rate of its growth.
- Stem thickness/diameter was measure using precision pocket Vernier caliper (150mm, Format, Wuppertal, Germany), the measure was done on the marked spot (shoot tip) of the previous week of the stem length/growth.
- Total number of leaves on each plant were counted on the last week of the experiment.

At the generative stage, we recorded weekly data on the following plant parameters:

- Number of clusters.
- Total number of fruits.

Data on leaf, SPAD index was measured using SPAD 502 Plus Chlorophyll meter a week after the seedlings were transplanted. Measurement was not randomly but it was calculated based on the following parameters:

- The total number of leaves,
- The part of the leaf that is closest to the stem and closest to the leaf tip.
- The leaf on the bottom and the last leaf of each plant were measured and the last leaf on each plant. However, the other leaves were measured after the preceding third leaf from below.

4.6. B= Czech university of Life sciences (CULS/ ČZU) greenhouse

The pot experiment with tomatoes was established 5th of May 2015. Into the 6 L pots was weight 3.933 kg of soil (82 % of moisture) and 1.667 kg of dried sand. The final ratio of dry soil and sand was also 2:1 (5 kg of dry material). Soil was mixed with sand to get the substrate with better air conditions as well as low content of nutrients. The soil was taken up from control treatment of

the long-term field experiments at Humpolec site, which belongs to Crop Research Institute in Prague – Ruzyně. The site characteristics are mentioned in table 1.

Table 6. Characteristics of location, where was taken up the soil for the pot experiment

Parameter	**Value**
GPS location	49°33'15" N; 15°21'02" E
Altitude (m above sea level)	525
Average yearly temperature (°C)	7,0
Average yearly rainfall (mm)	665
Soil classification	Cambisol
Soil texture	Loamy-sand
pH value [1)]	5,1
P (mg/kg)[2)]	77
K (mg/kg)[2)]	238
Ca (mg/kg)[2)]	1625
Mg (mg/kg)[2)]	112

[1)] Determined with 0.01 mol/L $CaCl_2$, 1:10 soil:extractant (modified after Minsany et al, 2011)

[2)] Determined using Mehlich 3 extract (Mehlich, 1984)

Whole experiment was realized in the climate chamber of greenhouse at Czech University of Life Sciences in Prague. The temperature was set up to 25 °C and the air humidity on 55 %. In total, 20 pots were established. One two weeks old tomato plant (variety *Mobile*) was planted in each pot. The treatments mentioned in table 2. Were established for the purpose of this experiment, all in four replications.

Table 7. Experimental design and concentration of stock solution

Var.	Biostimulant	Effective substance	Stock solution
1	Control	-	-
2	RhizoVital	Bacillus amyloliquefaciens (FZB 42)	14,0 ml/l
3	Trianum	Trichoderma harzianum (OMG 08)	4,7 g/l
4	Proradix	Pseudomonas sp. (DSMZ 13134)	5,5 g/l
5	CombiFect A	T. Harzianum+ B. Subtillis + Zn + Mn	4,0 g/l

The biostimulants were applied always in the form of stock solution (in amount recommended by producers). Five milliliters of stock solution were applied always on the roots by planting and the remaining 20 mL of stock solution broad on the soil surface immediately after planting. The pots were watered on 60 % of water holding capacity based on the pots weight. Pots were randomized each 14 days. On 12th May, whole experiment was protected against pests using Perfection (0.01 % solution). The harvest of mature fruits started on 12th June and finished subsequently on 17th September. To this date all fruits were harvested, including immature as well as the remaining above ground biomass.

4.7. Analytical methods and vegetation tests

4.7.1. Vegetation tests

Plant height was measured three times (26.6., 17.7. and 14.8.). Mature fruits were harvested at following days: 15., 22., 27. and 29.7., 4., 6., 10., 12., 17., 21., 28. and 31.8., 9. and 17.9. Fruits were always weighed and dried (50^{o}C). After determining the dry mass weight, fruits were further fine grounded for further analysis. The above ground biomass without fruits and soil samples were taken up at harvest as well. Above ground biomass was weighed and the soil samples were sieved through the 5 mm mesh and subsequently analyzed.

4.7.2. Determining of substrate pH value

For the determination of pH value, 5 g of air-dried soil (< 2 mm) was weighed. To this soil was applied 50 ml of demineralized water and the solution was shaken for one hour on horizontal shaker (120 U/min). Thereafter the solutions were lwft for one hour to stabilize and then measured directly to obtain pH value using the pH meter "HANNA Instruments, HI 991 300".

4.7.3. Determining of nutrients content using CAT extraction procedure

The content of selected nutrients bioavailable form was determined using CAT extraction (European norm EN 13651). This norm is describing the procedure to determine the content of bioavailable nutrients in soil with calcium chloride and diethylentriaminpentacetic acid (DTPA). This norm is not recommended for determining of bioavailable Calcium, because of $CaCl_2$ as a part of extracting solution. Fresh soil samples (< 2 mm) were extracted with the solution of 0.01 mol/L CaCl2 and 0.002 mol/L DTPA in the ratio 1:10 (soil: solution). After 1 hour of shaking (horizontal shaker, 120 U/min), samples were filtered, and the extracts measured. Tho content of ammonium and nitrate nitrogen was measured spectrophotometric with SKALAR SAN^{PLUS} SYSTEM instrument. Optical emission spectrometer with inductively coupled plasma (ICP-OES) was used to measure the content of phosphorus and micronutrients and the Atomic absorption spectrometer (AAS) to determine the potassium and magnesium content. All results were later calculated on mg/kg of dry soil.

4.7.4. Determining of bioavailable calcium content in water extracts

Extraction was realized using the procedure described by Luscombe (1979). To the 10 g of air-dried soil samples was applied 100 mL of demineralized water. Samples were shaken 1 hour (horizontal shaker, 120 U/min) followed by filtration. Extracts were then measured with ICP-OES.

4.7.5. Analysis of tomato fruits

Mature tomato fruits were fine grounded (<2 mm). The aliquote 0.25 g of fine milled material was weighed into the tubes for microwave digestion. Samples were digested in the environment of nitric acid and hydrogen peroxide (7 + 2 mL). Obtained solution was diluted with deionized water to 25 mL and these extracts were measured for the selected macro- and micronutrients content by ICP-OES (P, Mg, S and micronutrients) and AAS (Ca, K).

4.7.6. Determining of total nitrogen content in tomato fruits

The content of nitrogen was determined using method after Kjeldahl (ČSN 46 1011-18). For the extraction was weighed 0.500 g of dried, fine milled tomato fruits. These samples were mineralized with concentrated sulfuric acid with selenium added as a catalyst. The time of mineralization was 1 hour and the temperature 400 ^{o}C. Obtained mineralization were measured with Gerhardt Vapodest 50s machine.

4.8. Statistical analysis

Collected data from Uppsala were subjected to a one-way analysis of variance (ANOVA) to check the significance level of the various treatments on the plants in RCMDR program. Tukey's t test compares the means of each treatment to the means of every other treatment. We used 95% confidence level.

Descriptive statistics of the CULS dataset were realized using Microsoft Excel 2016. Further statistical evaluation as A-NOVA and Tukey test were analyzed in STATISTICA 12 (StatSoft, 2018)

CHAPTER 5

5. RESULTS

The results are divided into two parts like the method and materials since there are from different experimental set up with different parameter analysis.

In the SLU, the parameters were broadly categorized under vegetative and the generative growth in the analysis to test the effective of the various treatments.

Note on the results in SLU

The Blomstra treatment was used as only reference to the under-study treatments but not part of the under-study treatments. The Blomstra is an organic fertilizer.

5.1. Vegetative growth

5.1.2. Stem thickness

No significant difference was measured between control and bacteria solution during the experiment (figure 3.). The same was true in the case of silage 7.5 g and silage + bacteria fermentate treatments. Silage + bacteria fermentate showed statistically same values.Silage 15 g had significantly thicker stem since April 18th than control and bacteria solution.

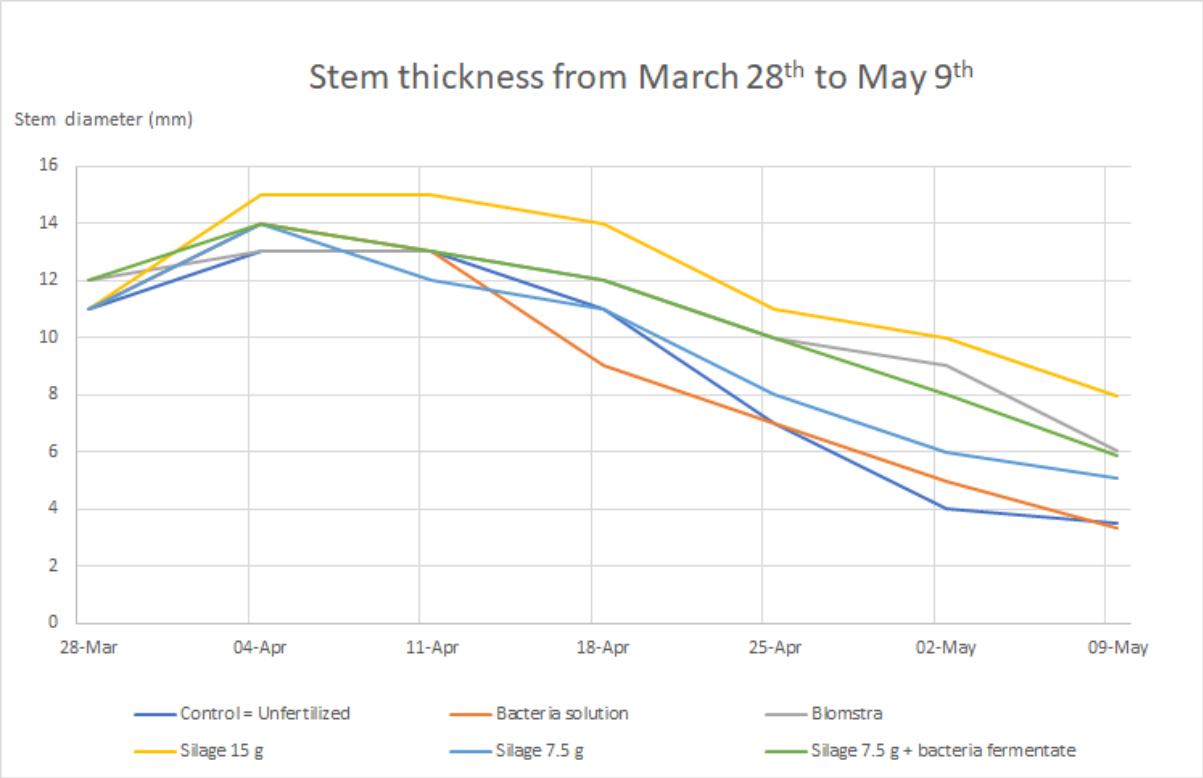

Figure 3. Thickness of the stem week by week. Until 18th of April the values were statistically the same, after that a significant difference was recorded later. At the end of the measurements silage 15 g showed significantly thicker stem than control and bacteria solution.

5.1.3. Stem length

The growth of the stem was measured weekly (figure 4.). The measuring point start from the top/tip of the last cluster of the plant measure previously, marked with a pen marker. During the experiment, there was no significant difference between the control and bacteria solution treatment, neither between silage 7.5 g and silage + bacteria fermentate treatments.

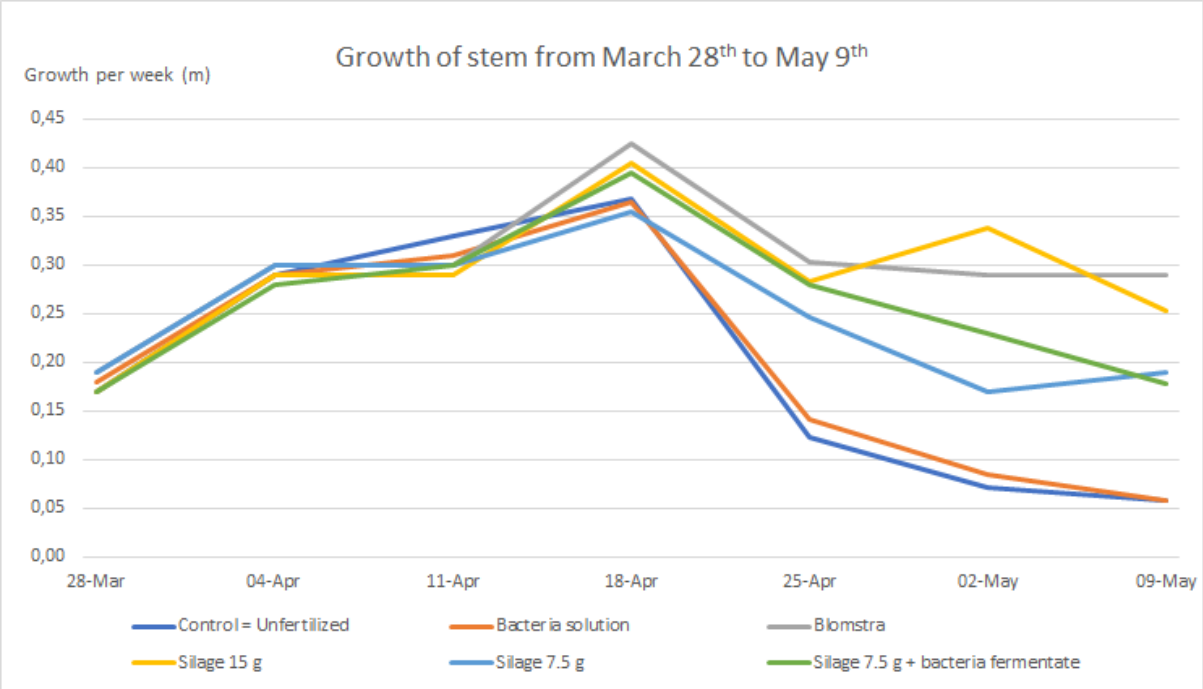

Figure 4. Growth of the stem week by week in the different treatments. The first significant differences were measured on the 25th of April. Before this, the plants showed similar pace in stem growth.

5.1.4. Plant Height

Total height of the plants was different at the end of the experiment (*Table 8*). The highest growth rate was recorded in the plant treated with only Blomstra: 2.58 m. The treatment with 15 g of silage was the next highest at the end of the study with 2.49 m growth. The treatment with 7.5 g of silage

and the bacteria fermentate showed a height of 2.31 m. The lowest growth rate was recorded in treatments with control which is 1.89 m and treatment with bacteria solution which was 1.90 cm.

Table 8. Total height of the tomato plants at the end of the seven weeks of the experiment.

Treatment	**Total height (m)**
Control = Unfertilized	1.89
Bacteria solution	1.90
Blomstra	2.58
Silage 15 g	2.49
Silage 7.5 g	2.23
Silage 7.5 g + bacteria fermentate	2.31

5.1.5. Number of leaves

Largest amounts of leaves were recorded in treatments Blomstra and silage 15 g and the lowest in bacteria solution which is shown on *Figure 5*. There was no significant difference between control and bacteria solution ($p=0.347$), neither between silage 7.5 g and silage + bacteria fermentate ($p=0.205$). Silage + bacteria fermentate had statistically the same amount of leaves as Blomstra ($p=0.112$) and silage 15 g ($p=0.205$).

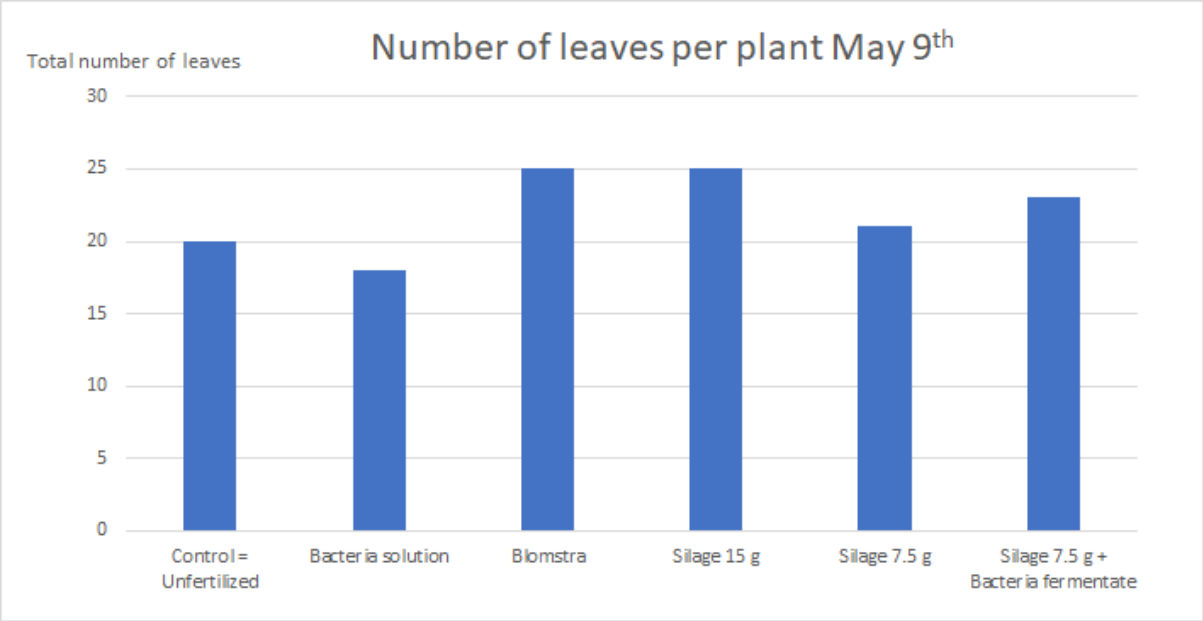

Figure 5. Total number of leaves at the end of the experiment. In the end bacteria solution and control had significantly less leaves than Blomtra and silage 15 g.

5.2. SPAD index of the leaves

No significant differences appeared in the SPAD index of the leaves until 2nd of May (Figure 6). Silage 15 g had significantly higher values than bacteria solution (p=0.043). On the 9th of May, sSilage 15 g (p=0.022) showed higher value than control plants. There was no difference between control and bacteria solution, and neither between silage 7.5 and silage + bacteria fermentate.

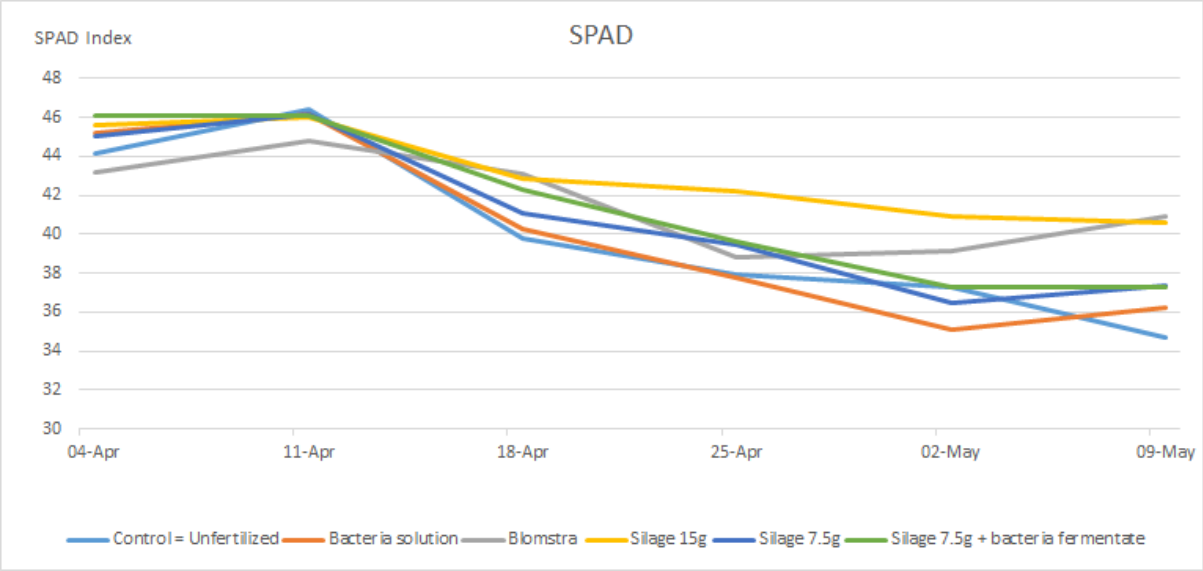

Figure 6. Result of the SPAD measurements week by week. Until 2th of May there were no significant differences between the measurements.

5.3. Generative growth

5.3.1. Number of clusters. Flower development.

Number of clusters per plant increased similarly in all the treatments from one cluster on 28th March (*Figure 7*). Silage 7.5 g with bacteria fermentate and Blomstra treatments had exactly the same results for number of clusters. There was a gradual increase on the first weeks. In the last measurement (May 9th) plants of Blomstra, silage 15 g and silage 7.5 g with bacteria fermentate went up to 7 clusters. Plants treated with silage 7.5 g did not develop any cluster during the last period (May 2th to 9th), remaining in six clusters. Control plants had an average of 5.5 clusters that did not increase in last period neither. However, bacteria solution treatment increased to a value of 5 clusters per plant.

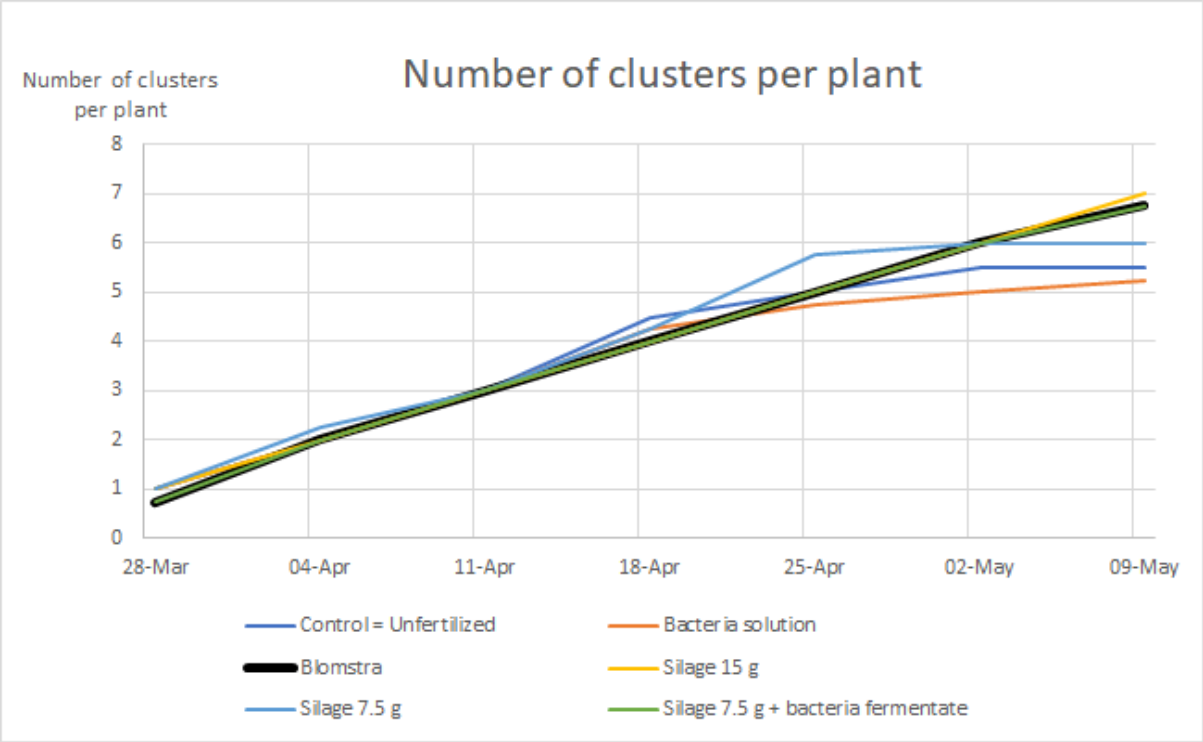

Figure 7. Number of clusters per plant.

5.3.2. Number of fruits

We measured the total number of fruits per plant, and analysed the data (Figure 8). During the experiment we did not notice significant differences between control and bacteria solution treatments. This was also true on the treatments with the treatment's silage + bacteria fermentate and silage 7.5 g. No significant differences appeared between silage + bacteria fermentate and Blomstra. The only significant difference between silage + bacteria fermentate and silage 15 g was measured on the last week, when silage 15 g had significantly higher number of fruits ($p=0.003$). Significant differences were recognized between bacteria solution and silage 15 g and between bacteria solution and Blomstra. Silage 15 g and Blomstra showed significantly higher number of fruits tan bacteria solution since April 25th. Also, Blomstra and silage 15 g showed higher values since that date than the control.

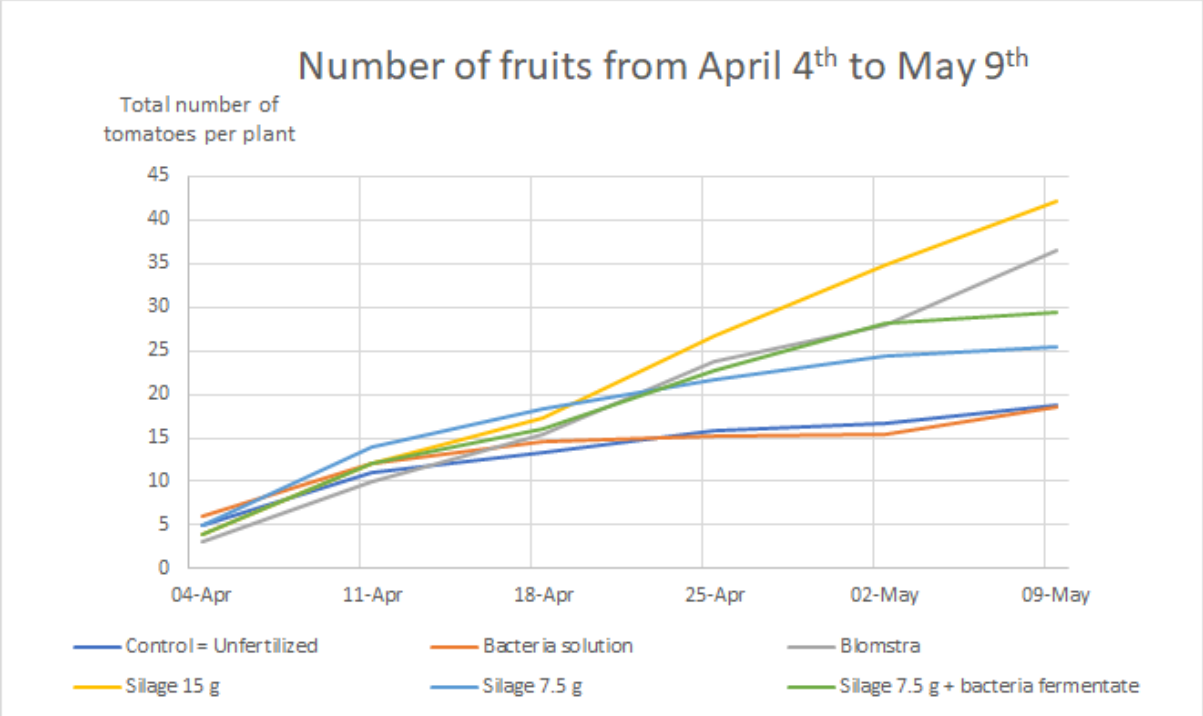

Figure 8. Total number of fruits in the treatments. Significant differences were measured since 11th of April. In the end silage 15g treatment showed the highest amount of fruits.

The results in CZU is based on nutrient analysis, they are categorized under macro and micro nutrients.

5.4. Results of tomato experiment in CULS.

Parameters measured.

Note to the tables

Attached letters shows significant difference of the treatments at $p \leq 0.05$: different letter means the significant difference between treatments according to Tukey test.

* All macronutrients in soil were analyzed by CAT extraction (EN 13651), only Ca content was determined using water extraction (see material and methods).

In the analysis of the tomato plants height, there was no significant difference between the control and the other treatments with biostimulants (Table 9). The microbial inoculant, that is *Rhizovital 42, Trichoderma OMG-08, Proradix* and *CombiFect A* were compared to the control treatments in the various weeks. Even though there was little bit increase in height of all plants treated with microbes but statistically, there was no significant difference.

Table 9: Average plant height (in cm)

Treatment	26.6.2015	17.7.2015	14.8.2015
control	72.75 [a]	76.00 [a]	76.63 [a]
Rhizovital 42	75.00 [a]	77.00 [a]	77.75 [a]
Trichoderma OMG-08	75.25 [a]	79.00 [a]	74.75 [a]
Proradix	71.13 [a]	76.50 [a]	74.88 [a]
CombiFect A	76.88 [a]	81.25 [a]	78.88 [a]

One the weight of fruits, there was no significant difference in the control and the microbe's treatments (biostimulants) in terms of fruit number, weight and dry mass ratio (Table 10). The control had less fruit weight and dry weight than the microbial inoculants but when the Tukey test was run to compare the means, there was no significant difference.

Table 10: Average number of fruits, weight and % of dry mass in above ground biomass

Treatment	Fruits per plant	dry weight (g)	dry mass (%)
control	7.0 [a]	27.8 [a]	7.7
Rhizovital 42	6.3 [a]	20.4 [a]	7.4
Trichoderma OMG-08	5.5 [a]	21.7 [a]	7.3
Proradix	5.3 [a]	21.0 [a]	7.2
CombiFect A	4.0 [a]	17.8 [a]	7.5

In the pH value and the content of macronutrients in the soil (Table 11), there was no significant difference between the microbe's treatments (biostimulants) and the control treatment. None of the treatments with the microbial inoculants and the control treatment showed a significant difference in the soil pH, the microbes failed to establish themselves to be able to effect a change in the pH of the soil. This was same in terms of Macronutrient availability in the soil, the control treatment was obviously not showing stronger growth during the experiment due to lack of nutrients.

Table 11: The pH value and the content of bioavailable macronutrients in soil (in mg/kg)

Treatment	pH_{H2O}	$N\text{-}NO_3$	$N\text{-}NH_4$	P	K	Ca*	Mg	S
control	5.92[a]	7.33[a]	3.39[a]	9.68[a]	22.60[a]	17.39[a]	71.19[a]	1.96[a]
Rhizovital 42	5.82[a]	5.02[a]	3.41[a]	10.56[a]	21.66[a]	17.13[a]	78.19[a]	2.13[a]
Trichoderma OMG-08	5.84[a]	3.98[a]	2.52[a]	10.41[a]	23.30[a]	17.43[a]	78.86[a]	2.23[a]
Proradix	5.87[a]	3.73[a]	1.18[a]	11.06[a]	22.91[a]	17.12[a]	67.58[a]	1.96[a]
CombiFect A	5.84[a]	4.22[a]	1.63[a]	10.98[a]	20.41[a]	15.22[a]	76.25[a]	2.18[a]

With the bioavailability of micronutrients in the soil (see Table 12), there was no significant difference in all the treatment except between the control and Proradix in terms of manganese mobilization. The Proradix, which contains *Pseudomonas,* strain FZB 13134 was able to chelate manganese to improve the soils biota, but the control treatment could not. This was probably a result of the bacteria's ability to mobilize such a micronutrient.

Table 12: The average content of bioavailable micronutrients in soil (in mg/kg)

Treatment	Fe	Cu	Mn	Zn
control	94.2 [a]	2.3 [a]	96.1[a]	1.3 [a]
Rhizovital 42	105.2 [a]	2.4 [a]	111.6[ab]	1.3 [a]
Trichoderma OMG-08	103.1 [a]	2.4 [a]	110.3[ab]	1.4 [a]
Proradix	113.8 [a]	2.5 [a]	118.5[b]	1.4 [a]
CombiFect A	100.8 [a]	2.2 [a]	105.0[ab]	1.3 [a]

In the macronutrient content in the tomato fruit (Table 13), there was only a significant difference in terms of sulfur (S) content in control treatment and CombiFect A. There were some microbial activities in terms of S mobilization to the plant, which manifested in the mature tomato fruit. CombiFect A, which is a combination of several microbes and Trichoderma OMG-08 were able to help in S mobilization.

Table 13: The average macronutrients content in mature tomatoes fruits (all in mg/kg)

Treatment	N	P	K	Ca	Mg	S
control	19535[a]	2314 [a]	28839[a]	650[a]	1243[a]	886[a]
Rhizovital 42	18692[a]	2452[a]	28399[a]	723[a]	1243[a]	919[ab]
Trichoderma OMG-08	16817[a]	2349[a]	26991[a]	726[a]	1154[a]	866[a]
Proradix	20287[a]	2269[a]	30621[a]	733[a]	1205[a]	1000[ab]
CombiFect A	18480[a]	2514[a]	29073[a]	669[a]	1215[a]	1044[b]

In macronutrient uptake by the tomato (Table 14), there was a significant difference between Control and treatment with CombiFect A in terms of nitrogen uptake, where the N content in fruit was lower in CombiFect A in comparison to Control. The treatment with CombiFet A, which is a

combination of several bacteria was not able to take up the nitrogen sooner than the plants probably.

Table 14: The average macronutrients uptake by mature tomatoes fruits (all in mg)

Treatment	N	P	K	Ca	Mg	S
control	535 [a]	64 [a]	797 [a]	18 [a]	35 [a]	25 [a]
Rhizovital 42	389 [ab]	50 [a]	587 [a]	15 [a]	26 [a]	19 [a]
Trichoderma OMG-08	367 [ab]	51 [a]	588 [a]	16 [a]	25 [a]	19 [a]
Proradix	424 [ab]	48 [a]	641 [a]	15 [a]	25 [a]	21 [a]
CombiFect A	325 [b]	44 [a]	512 [a]	12 [a]	21 [a]	18 [a]

In micronutrient content in mature tomato fruits (Table 15), there was a significant difference between control treatment and treatment with *Trichoderma* in terms of Iron (Fe). There was also a significant difference between treatment with *Trichoderma* and CombiFect A in terms of Zn content in the tomato fruit. The treatment with *Trichoderma* fungi was probably able to immobilize Fe through its fungi hyphae to the plants and this resulted in the significant difference between that treatment and the control treatment. This also resulted in a significant difference between that treatment and treatment with CombiFet A in terms of Zn availability to the plants. The fungi hyphae were more robust in the Zn mobilization than the chelating activities of the bacteria, this resulted in the significance during the fruit analysis.

Table 15: The average micronutrients content in mature tomatoes fruits (all in mg/kg)

Treatment	Fe	Cu	Zn	Mn	Al
control	43.03 [a]	8.11 [a]	13.86 [ab]	10.59 [a]	13.27 [a]
Rhizovital 42	31.22 [ab]	8.26 [a]	13.62 [ab]	11.18 [a]	9.75 [a]
Trichoderma OMG-08	27.58 [b]	6.63 [a]	11.39 [a]	11.33 [a]	9.41 [a]
Proradix	31.17 [ab]	7.76 [a]	14.88 [ab]	10.64 [a]	8.80 [a]
CombiFect A	32.66 [ab]	7.37 [a]	16.42 [b]	11.18 [a]	11.51 [a]

In terms of micronutrient uptake by mature tomato (Table 16), there was only a significant difference in control treatment and treatment with *Trichoderma* and between *Trichoderma* and CombiFect A in terms of Iron (Fe) uptake. The Trichoderma could not help in the uptake of Fe by the plant through its hyphae, which should have been the same case with the CombiFect A. This resulted in the significant difference recorded in Fe uptake by the mature fruits during micronutrient analyses of the tomato fruits.

Table 16: The average micronutrients uptake by mature tomatoes fruits (all in mg)

Treatment	Fe	Cu	Zn	Mn	Al
control	1.23 [a]	0.23 [a]	0.38 [a]	0.29 [a]	0.38 [a]
Rhizovital 42	0.65 [ab]	0.17 [a]	0.28 [a]	0.23 [a]	0.19 [a]
Trichoderma OMG-08	0.60 [b]	0.14 [a]	0.25 [a]	0.25 [a]	0.21 [a]
Proradix	0.66 [ab]	0.16 [a]	0.31 [a]	0.22 [a]	0.18 [a]
CombiFect A	0.58 [a]	0.13 [a]	0.29 [a]	0.20 [a]	0.20 [a]

CHAPTER 6.

6. Discussion

6.1. Vegetative growth

Biostimulant such as *Pseudomonas spp.* produce phytohormones like cytokinin and IAA (Indole-3-acetic acid) which promote plant growth (Vessey 2003). To determine the plant vegetative growth a weekly measurement was taken on the following: stem thickness, stem growth, total number of leaves and chlorophyll content. Control and bacteria fermentate treatments showed the same results. There was no difference between the results of the control and the result of the bacteria fermentate in the early weeks of the experiment, even though the bacterial is well known for their ability to influence plant growth. However, it could be dependent on species specificity that is the type of species of *Pseudomonas spp*. and the amount of organic matter in the soil. The amount of organic matter influences the bacteria's biochemical activities, the bacteria ability to chelate or mobilize nutrients to the plant is based on the amount of organic acid it is able to produce and release to attract positively charged elements (cation) calcium, iron, copper, zing etc (Kpomblekou-A and Tabatabai 1994; Yu et al (2012). This could have affected bacteria ability to function as expected during the experiment. In addition, the amount of organic compound in the soils used could affected the cation exchange capacity of the soil and the bacteria is less active than expected.

Most scholars have focuse on the importance of the organic matter to the activities *Pseudomonas* spp. McCall and Height (1981), also emphasized that the presence or absence of organic matter in the soil could limit the activities of the bacteria. The bacteria have the ability to produce ammonia from the organic matter for its host. The lack of enough organic matter in the soil used for the experiment made it difficult for the bacteria to increase the nutrient content and to enhance the plants growth. This absence of organic matter affected the activities of the bacteria (Sarathchandra 1978; McCall and Height 1981). This lack of establishment of the bacteria within the tomatoes root system made it fail trigger phytohormones such as gibberellic acid and auxin to stimulate the tomato shoot elongation making the plant to grow tall (Hamza and Suggars 2001). Statistically there was no significant difference when we run the Tukey's test on the on the length of the stem between the control and bacteria fermentate treatment. This makes the study contrary to the results

obtained by (Domenech et al., 2006) on similar experiment where the bacteria species were able to stimulate growth in height, leaf sure face area and number of leaves on the tomatoes plants.

6.1.2 Stem thickness

On stem thickness, there was some prominence exhibited between treatment silage15 in terms of stem thickness and stem height (length of stem) there was significant difference in the tomato plants treated with silage 15 and the control and the bacteria fermentate. These differences manifested in on the 18th April and on the 25th of April. This could be attribute to the amount of N content which is 15g mixed with silage which has the capacity to release the nutrient to the tomatoes in a slower pace. The organic matter in that treatment are able to facilitate tomatoes nutrient uptake and enhances growth and development. The growth and development response are not only connected to the nutrient content in the organic substances but the biochemical activities. And there is also an interaction between the "transport membrane" and the growth regulation in the plant system (Canellas et al., 2015). The slower pace in release caused the plants stem thickness and the height inability to show any significant difference in the early weeks of the experiment. This significant difference was as a result of the positive impact of the the organic substances in that treatment. The organic matter helps in improving the soils physical properties and increasing the amount of nutrient available to the plant (Chen and Aviad, 1990; Stevenson, 1994; Akinci et al., 2009; Nazli et al. 2016), which is in line with studies conducted by Nazli et al. (2016), cited by Materechera and Salagae (2002) as well as Khan et al. (2008) to buttress their findings on how the diameter of a corn cob is influenced by the organic matter. They stated that, the diameter of the corn cob is highly dependent on the amount of organic material in the soil and in combination with inorganic fertilizer in maize plant. This was contrary to the studies conducted by (Ali et al. 2012; Nazli et al. 2016) on maize plant, they also concluded that organic material application has no significant effect on the increase in plant height.

SPAD index has a close relationship with the chlorophyll content of the leaves, the N content of the plant and the yield. In this experiment, the SPAD reflected in the amount of yield (total number of fruits) on each plant. The following treatment; Blomstra and silage 15 g showed significantly higher SPAD index and also significantly more fruits than the control treatment even though the Blomstra treatment was not part of the under studied. It is in line with the studies conducted by Materechera and Salagae (2002) in Khan et al. (2008) that the diameter of the corn cob was

influenced by organic material, when applied with inorganic fertilizer in maize plant (Nazli et al. 2016), since corn cob has a direct relationship with the grains on the maize plant. Even though Blomstra is not chemical fertilizer but rather an organic one, they all have some common feature they share.

Research conducted by Båth, (2009) indicated that plants respond positively in biomass production when fertilized with silage and manure as well as N. This was the case in the tomato plants, which were treated with silage 15 (15 g N). They had higher vegetative growth than other treatments among studied. This is because those treatment had double amount of N than the treatment silage 7.5 (7.5 g N). This result in slow pace affected the nutrient availability to the plants and hence affected their vegetative growth could be caused by abiotic factors. I observed that around the walls of the greenhouse had little bit of a different temperature even though there were plants that serves as a protection, but it can never be underestimated on the effect it can have on the plants. Hjeljord et al. (2000), indicated the importance of temperature on nutrient availability to plants in a greenhouse. They realized a change in temperature in some parts of the greenhouse had affected the activities of the microbes (Trichoderma) on *Botrytis cinerea* and *Mucor piriformis* on greenhouse strawberry. This is in also in line with the works of Ghorbanpour et al. (2018), who concluded that cold stress or temperature has negative effect on tomato plant. The cold temperature can cause deleterious effects on the plants and also impact negatively on their growth.
In the last weeks the experiment, the growth rate in treatment with silage (15 g N) and treatment silage (7.5 g N) did not show any significant difference. However, the Blomstra treatment had higher vegetative growth than silage 7.5 g because of the liquid nature of the Blomstra, which was applied daily. This make it easier for the plants to pick up the nutrients for their growth.

6.2. Generative growth

The most common knowledge known about the relationship between the *Pseudomonas* bacteria and the plants is their ability to induce positive effects on plant growth and development through nutrient uptake (Sahar et al. 2013; Bartolini et al. 2017). Studies of Kloepper (1991); Bowen and Rovira (1999) shows that *Pseudomonas* bacteria can increase crop yield, control root pathogen and increase plant resistance. However, in this study, statistically there was no significance difference between the control treatment and treatment with bacteria solution.

In the first week, that is on the 4th of April, there was no significant difference in the analysis on the fruit formation, when the Tukey's test run to compare control and bacteria solution. This can be attributed to the fact that the plants were investing in their growth than to their development coupled with the low lighting regime. This was due to the winter season, which comes with low light righting regime and early spring. Studies conducted by Heuvelink (1994) indicates that low light regime may favour vegetative growth over generative growth. The flower initiation, pollen viability and fruit formation can be affected by the low light regime caused by winter and early spring around the greenhouse. From the middle time of the experiment until the end, the bacteria did not show any significant difference with the control. This can be attributed to observations made by Kremer (2007), who indicated that some species of *Pseudomonas* can cause retardation in plant growth and development depending on crop cultivar, which is based on their symbiotic relationship and the present of some deleterious microbes in the soil. This could be a factor as to why there was no significant difference.

The soil used in the experiment might have contributed to that due to the lack of nutrients. Studies conducted by (Peer et al. 1990; Bowen and Rovira 1999) indicates that *Pseudomonas* fluorescens is unable to mobilize nutrient in and around the rhizosphere if the soil lacks nutrients due to repeated cropping of same cultivar (for example barley). Moreover, the soil used in the experiment was not well analysed, to ensure that it is free from "deleterious rhizosphere microorganisms". It can retard the development process of the plant and interfere with the bacteria nutrient mobilization (Suzlow and Schroth 1982; Bowen and Rovira 1999).

In our entire period of studies, we did not find any statistically significant difference between silage 7.5 g and silage + bacteria fermentate. However, we found some slight difference in the fruit and flower development. This could be attributed to the fact that the silage 7.5 g had less amount of N that releases to the plant slowly. This affects the plant ability to develop faster as compared the silage + bacteria. The bacteria are known to have the ability to mobilize nutrient to the plant. This probably resulted in the slight improvement in the flower and fruit development than that of silage 7.5 g. *Pseudomona*s bacteria helps plants in uptake of nutrient, which improve their growth and development (Adesemoye and Kloepper 2009).

6.3. Discussion of the second experiment at CULS

The results too were not promising as anticipated, the microbes did not make much impact if not more redundant during the experiment.

Parameters analyzed were vegetative growth and nutrient content in the soil as well as the fruits.

In the plant height analysis, it was overserved none of the biostimulant were able to influence the plants growth in height. There was no significant difference between the various microbe's treatment and the control treatment. This is contrary to the well-known suggestion that biostimualt has the capacity to stimulate growth hormones in plant. The boistimulants used in this experiment were *Trichoderma, Pseudomonas sp, Bacillus Amyloliquefaciens* and CombiFect A. These species of microbes are able to produce phytohormones, which regulate several plant tissues-related activities, ranging from "stem elongation, root initiation and tissue differentiation", plant defence and stress tolerance. One important phytohormones is gibberellic acid stimulates shoot elongation making the plant to grow tall or increase in stem height (Hamza and Suggars, 2001).

Bulgari et al., (2015), held similar view that, these substances/microbes are able to act or stimulate growth and development only if they are able to penetrate the plant tissue. But they were quick to add that, the success of these microbes and their rate of work depends on its permeability, weather conditions and other factors like the soil condition. This could have been the case that made the microbes failed to response in the tomato plants growth in height during the experiment. Root exudates is very vital in the activities of microbes, in terms of hyphae growth and ability to chelate nutrient for the plants. It was contrary to the findings of El-Komy (2005), where *Pseudomonas fluorescens* and *Bacillus megaterium* strains were able to mobilize P for wheat plant and thereby facilitating their vegetative growth.

Furthermore, those abiotic factors could have caused the inability of the microbes especially (*Pseudomonas spp, Bacillus subtilis etc*) to produce indole-3-acetic acid (IAA). They need a good temperature regime and an optimum level of humidity in order to be able to act in the rhizosphere. Vessey (2003), indicated that the IAA plays a vital role in plant growth and development and be beneficial or deleterious depending on the environmental conditions.

One the fruits weight, the microbes failed to make impact, and therefore it was found that there was no significant difference between treated with microbes and the control treatment in terms of

their biomass weight. The success of a treatment gives it an advantage to have more biomass weight than the other. Many studies have postulated that microbes have the ability to increase the plant growth and development, and growth and development are intertwined with the amount of biomass a plant has. Colla et al. (2015) observed an improvement in growth parameters of lettuce in leaf length, number of leaves, shoot after the inoculation of some *Trichoderma* strain. Molla et al. (2012), in their research found a huge increase in vegetative growth in tomato plants when some species of biostimulant (*trichodermin sp*.) were inoculated.

However, the plant defense system can harm the success of the microbial inoculant. The issue is that the plant has a sophisticated system in its defense pathway. It could recognize the microbial inoculants as effectors then trigger an immune response to suppress the growth and activities of the microbes in the rhizosphere. To support this observation or assertions, the works of Lekfeldt et al. (2016), on spring wheat indicated plants treated with microbial inoculants failed to influence the growth of the wheat plants and thereby unable to influence the aboveground biomass. But, the control treatment, which has no microbial inoculant was successful in the increase in aboveground biomass. They concluded that, there was no effect on the plant's aboveground biomass, when microbial inoculants were applied. El-Komy (2005), holds a contrary view on this, He observed a higher dry weight and an increase in yield in wheat plants treated with *Azospirillum lipoferum* 137 and Ca_3PO_4 even though he observed a lower content of P than those that were fertilized. He then suggested that, *Azospirillum* could have a several mechanisms in helping plants to mobilizing nutrient. This may include nitrogen fixation and hormonal effect activities. Similar observation was made by Nassal et al. (2018). In their experiment with different *Pseudomonas spp*. they concluded that treatments with a RU47 a strain of *Pseudomonas ssp.* on tomato increased the P availability in the soil, which lead to improve plant growth like higher stem diameter, bigger leaf area and "3-folder" shoot biomass which was due to the phosphatase activities in the rhizosphere of tomatoes.

6.3.1. Macronutrients assessment.

On the content of bioavailable macronutrient in the soil, there was no significant difference between the control and the microbial inoculants after the soil analysis (see table 11). This means that the microbial inoculant did not function as expected, but with the control treatment it was

obvious that macronutrients wouldn't be found in that since nothing was added. This was in line with studies conducted by Lekfeldt et al. (2016), where some biostimulants of bacteria (Proradix and RhizoVital 42) and a fungal product (Biological fertilizer DC) were investigated. These microbial inoculants failed to facilitate P mobilization in the rhizosphere of the wheat plants. They gave several reasons that could have caused that; "(i). the soil P level may not have been sufficiently low to promote the up-regulation of enzymes involved in P solubilization, (ii). The amount of P released may have been taken up by the introduced microorganisms without subsequent release to the soil within the time frame of the experiments and finally (iii). Another reason is the limited proliferation of the introduced microorganisms in soil due to competition with native microorganisms".

This is contrary to the studies conducted by Yu et al. (2012), where they observed an increase in P and N in the rhizosphere of the soil used for walnut seedlings treated with phosphorous solubilization bacteria and nitrogen fixation bacteria *Pseudomonas chlororaphis* and *Bacillus megaterium*. However, with regards to soil pH, single inoculant of the microbes; *P. Chlororaphis, Bacilus megaterium* and *Arthrobacter pascens* (*A.pescens*) did not show any significant difference in the soil pH. Same results were observed in the treatment that contains the combination of all the treatment, which is in line with the results we obtained in Table 13.

On table 14. (on the macronutrient uptake in the tomato) there was only a significant difference between CombiFect A and the control treatment in N uptake in the tomato plant. There was a decrease in the nutrient uptake with CombiFect A. This was in contrary to the works of Yu et al. (2012), who found that a mixture of microbial inoculants was able increase N uptake in walnut seedlings. They concluded that the mixture of the microbial inoculants culture allowed their components to interact with each other synergistically, thus, stimulating each other through physical or biochemical activities.

6.3.2. Micronutrients assessment.

The content of bioavailable micronutrients in the soil and micronutrients content in mature tomatoes fruits is mentioned in Table 12. and Table 15., respectively. The microbes could not mobilize the needed micronutrient as expected. The only significant difference was recorded in the amount of micronutrient in the soil between Control treatment and treatment with *Proradix* in

Manganese (Mn) mobilization. In addition, micronutrients content in mature tomatoes fruits, there was only a significant difference recorded between Control treatment and treatment with *Trichoderma OMG-08* in terms of Iron (Fe) mobilization. A significant difference was also recorded between treatment with *Trichoderma OMG-08* and *CombiFect A* in the mobilization of Zinc (Zn). These was contrary to the studies conducted by Colla et al. (2015). They observed an indirect effect of the microbes on lettuce, melon and pepper. They observed an increase in micronutrient availability during the early days after transplanting. There was an evidence of growth stimulation which was triggered by these micronutrients. The increase in root surface area facilitate the capture of theses positively charge elements by the root system. This was contrary with the works of Günes¸ et al. (2009); Ruzzi and Aroca (2015), where the application of a microbe *Bacillus* in strawberry was able to increase the concentration of micronutrients in the plant fruits and leaf.

CHAPTER 7

Conclusion

Reducing the use of fertilizers and changing mineral fertilizers to organic ones are two main goals for food production worldwide.

In this experiment, the obtained result contradicted our prediction. In our hypothesis we assumed that Biostimulant (bacteria fermentate) was going to improve plant growth as compared to the control (unfertilized) or comparing to the silage 7.5 and silage+bacteria fermentate treatments. The bacteria were never significantly better in the measured indicators, so we can indirectly say that the use of *Pseudomonas*, strain MS100 did not help in mobilizing the unavailable nutrients in the soil for the plants.

We also assume another hypothesis, that biostimulants have the ability to mobilize both macro- and micronutrient to the tomato plants. This was confirmed only in few cases, where the *Pseudomonas* application increased the mobilization of manganese in soil and CombiFect increased the content of sulfur in plants. However, some effects of biostimulants were negative – *Trichoderma* application decreased the S, Zn and Fe content as well as Fe uptake by tomato fruits. Furthermore, CombiFect A application led to decrease of nitrogen uptake with tomato fruits.

Based on the outcome of our experiment, we will recommend for a further research on the kind of fertilization to improve vegetative and generative growth in tomato plant as well as nutrient analysis. The species of bacteria should be carefully analyzed before selection.
Another concluding remark is that the whole idea of bionstimulant has been overrated by the scientific community and most journals, products developers and distributors are more interested in publishing results that show positive outcomes of biostimulants. It could lead to overestimated look on the role of biostimulants function on yield and quality parameters.

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