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THE INFLUENCE OF BIOSTIMULANTS ON MAIZE'S YIELD AND SOIL NUTRIENT RELEASE

MASTER THESIS

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Objectives:	1) to investigate the effect of biostimulants on maize growth and yield 2) to find out the effective biostimulants treatments according to nutrient content nutrient uptake with maize aboveground biomass

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DECLARATION OF ORGINALITY

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SUMMARY

The modern agricultural practices have been introducing towards organic, sustainable or environmentally friendly systems. These modern agricultural practices aim to reduce inputs without reducing the yield and quality. The adverse effect of these synthetic chemicals on human health and environment can only be reduced or eliminated by adopting new agricultural technological practices such as organic agriculture, sustainable agriculture or ecological agriculture. Biofertilizers are recognized as biological products including living microorganisms or different natural extracts. When they are applied to seed, plant surfaces, or soil, they promote growth by several mechanisms such as increasing the supply of nutrients, increasing root biomass or root area, and increasing nutrient uptake capacity of the plant. Biofertilizers can be used as complements to mineral fertilizers.

The screening pot experiment was set up to investigate the effect of biostimulants on maize growth and yield. Proradix, RhizoVital 42 (RV42), RhizoVital 45, MUCI, RV42 + MUCI, SuperFifty, NemaTec, LamVita, Biological Fertilizer OD and Trichoderma OMG were used as biostimulants to investigate the effect on maize growth and nutrient contents and uptake in both applications.

The positive effect of biostimulants did not occur in single biostimulants application. Control (soil only) treatment was the best one in biostimulants only applications, which means that application only biostimulants into the soil was not effective. However, some positive effect was obtained in the combined application of RP with biostimulants treatments. NemaTec treatment, which belongs to the seaweed extracts group, was the effective one within the combined application of RP with biostimulants. RP + NemaTec provided better results in plant height, biomass weight, and uptake of P, Mg, Ca, Mn when compared to all biostimulants application in our experiment. On the other hand, the negative result of maize growth and yield was observed with MUCI, where were also usually found the lowest contents and uptake by maize aboveground biomass

Keywords: Biostimulants; Bofertilizer; Maize; Rock phosphate; Sustainable agriculture

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CHAPTER 1
1. INTRODUCTION

Agriculture is one of the human activities that contribute to increasing the number of chemical pollutants from the excessive use of synthetic chemical fertilizers and pesticides. The improper use of these chemicals causes further environmental pollution with potential risks to human health. Nowadays, the use of fertilizer in agriculture is higher and expensive. These applied fertilizers are released to the environment. The global population is increasing year by year. Thus, Farmers around the world will need to increase crop production by increasing the amount of agricultural land to grow crops or by enhancing productivity on decreasing agricultural lands through fertilizer and irrigation. Therefore, the use of fertilizer will be needed more and more to obtain a higher yield.

Nowadays, agricultural production systems are facing increasing competition with other sectors for limited natural resources around the world. Improper management practices, changing climatic and weather conditions steadily reduce the availability of these resources and their quality. To overcome this situation, the agriculture sectors must improve their sustainability performance and adapt to the impacts of climate change.

The application of fertilizer is to ensure plant growth by providing certain deficient nutrients. They also have many other advantages such as the cheaper source of nutrients, higher nutrient content, easily soluble in soil solution and readily available to plant. Besides, it is required in less amount and used easily, which makes it more acceptable than organic fertilizer. The inorganic fertilizers are widely used in agricultural production as they can improve the yield of crops significantly. The use of fertilizers promotes crop production and improves soil fertility. Thus, the yield of crops is independent and ensure incomes. For this reason, using inorganic fertilizer in crop production is very popular around the world.

The industrial revolution and the green revolution which achieved the food demands of the growing population caused an increase in yield. But they also improved dramatically the use of mineral fertilizers in agricultural production. Less soil fertility is one of the important limitations in increasing agricultural production. However, inappropriate and the intensive use of inorganic fertilizers in agriculture for ensuring the world food security caused too many health problems and unrecoverable environmental pollution such as water pollution, air pollution and land degradation.

CHAPTER 2

2. SCIENTIFIC HYPOTHESIS AND OBJECTIVE OF WORK

2.1 Hypothesis

Agricultural practices should be safe for sustainable agricultural production to fulfill the need of growing population. Agricultural sector is one of the important factors contributing to environmental population such as soil, water and air population. Application of chemical fertilizers dramatically destroy our environment. Therefore, biostimulants application could be one of reasons to create safe and clean an environment because they have the potential to increase the health and productivity of plant life and steadily reduce the need to use chemical fertilizers.

2. 2 Objectives

The objective of the present study is to investigate efficient usage of biostimulants that will improve plant's nutrient uptake, plant growth and yield.

The present study was conducted with the following specific objectives:

1) To investigate the effect of biostimulants on maize growth and yield and nutrient uptake
2) To find out the most effective biostimulant treatment on this on local soil.

CHAPTER 3
3. LITERATURE OVEREVIEW

3. 1 Definition of biostimulants

Plant bio-stimulants, or agricultural bio-stimulants, include diverse substances and microorganisms that enhance plant growth. In past, various types of bio-stimulants have been defined based on source material, mode of action and other parameters by many authors (Calvo et al., 2014). The European bio-stimulants industry council (EBIC) defined plant bio-stimulant as "Plant biostimulants contain substance(s) and/or micro-organisms 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"(Anonym, 2020).

3. 2 The use of biostimulants in agriculture

For a sustainable agricultural vision, crops productions must be prepared with disease resistance, salt tolerance, drought tolerance, heavy metal stress tolerance, and better nutritional value. The negative effect of chemical fertilizers on human health and environment can only be reduced or eliminated by adopting new agricultural technological practices such as organic agriculture, sustainable agriculture or ecological agriculture. Most of organic fertilizers are primarily cost-effective, easily available from locality products than chemical fertilizers (Kumar et al., 2019).

Around the world, a large number of chemicals such as fertilizers, pesticides, herbicides, have been used in agricultural systems to achieve more production per unit area. However, using over doses than optimum or recommended of these chemicals and fertilizers leads to several environmental pollution such as soil, water, air pollution, reduced input efficiency, decreased food quality, resistance development of different weeds, diseases and insects, soil degradation, micronutrient deficiency in soil, toxicity to different beneficial living organism present above and below the soil surface, less income from the production, etc. (Chandini et al., 2019). Besides, there is also a challenge to meet the food demands of the world's growing population. Thus, we need to produce nutrition rich and chemicals free agricultural products for the human and animal consumption without destroying natural resources.

Fertilization is one of important ways to increase efficiency and obtain better quality of product recovery in agricultural activities. Chemical fertilizers mainly contain phosphate, nitrate, ammonium and potassium salts. Fertilizer industry is a potential source of natural radionuclides and heavy metals. Plants absorb these chemical fertilizers through the soil, they can enter the food chain (Savci, 2012). Furthermore, the use of chemical fertilizers in agriculture is one of the key sources and driver for greenhouse gases (GHGs) emissions from agricultural soils. Direct and indirect emissions of greenhouse gases from agricultural soil are determined by many factors such as rate of fertilizer and organic manure application, yield and area under cultivation. Direct emission sources especially are N fertilizers, crop residues, and mineralization process of soil organic matter. N_2O is produced by microbial transformations of nitrogen (N) in soils and animal waste and therefore often associated with N fertilizer inputs in agricultural system (Lenka et al., 2016).

Today, people are aware of harmful effect of nitrogenous fertilizers. It also contains carcinogenic substances. Nitrogen is widely used in agricultural production to achieve higher yield of crops because it is one of the limiting factors in crop growth and production. A significant amount of urea fertilizer applied to the field is converted into nitrate which has the potential to contaminate groundwater under special soil management and climatic conditions due to their high mobility. The significant amount of nitrate in drinking water can cause methaemoglobinanemia in infants and added risk factor in developing gastric and intestinal cancer. Many researches have shown that excessive use of nitrogenous fertilizers in agricultural production is one of the major sources of nitrate pollution in drinking water (Lenka et al., 2016).

In recent years, many researchers have reported that microbial inoculants can also play an indirect role on soil remediation and soil fertility. Nowadays, bioremediation is one of popular tools to restore contaminated areas, reforest eroded areas, and restore degraded ecosystems (Calvo et al., 2014). Microbial fertilizers are particularly environmental-friendly, non-bulky and cost-effective. Furthermore, they also play a significant role in plant nutrition. Some other agricultural technologies and management practices such as integrated nutrient management (INM), using slow release fertilizer or Nano-fertilizers, conservation tillage, cover cropping etc. can be adapted to supply balanced nutrients to plants. Fertilizers are very important for the crop growth, yield, quality parameters, even for soil health only when applied in optimum recommended dose or when used judiciously (Chandini et al., 2019).

Plant biostimulants, or agricultural biostimulants, involve diverse substances and microorganisms that enhance plant growth. The global market for bio-stimulants has been projected to reach $2.241million by 2018 and to have a compound annual growth rate of 12.5 % from 2013 to 2018. According to some publications, Europe was the largest market for bio-stimulants in 2012. According to the European bio-stimulants industry council (EBIC), in 2012, over 6.2 million hectares were treated with bio-stimulants in Europe (Calvo et al., 2014)

Using of microbial inoculants in agriculture has greatly increased during the past two decades (Hayat et al., 2010) because the public and private sector agricultural research and development communities try to solve the problems associated with modern agriculture. Microbial inoculants can be typically classified as biocontrol agents (also called biopesticides) or biofertilizers (Bashan and Holguin, 1998). Biofertilizers are recognized as biological products including living microorganisms. When they are applied to seed, plant surfaces, or soil, they promote growth by several mechanisms such as increasing the supply of nutrients, increasing root biomass or root area, and increasing nutrient uptake capacity of the plant. Biofertilizers can be used as complements to mineral fertilizers. Microbial inoculants especially involve fungi, free-living bacteria, and arbuscular mycorrhizal fungi (AMF). They are isolated from a variety of environments including soil, plants, plant residues, water, and composted manures (Calvo et al., 2014).

3. 3 Classification of biostimulants

Bio-stimulants are available in many formulations and with varying ingredients. Most popular bio-stimulants are humic substances (humic acid and fluvic acid), protein hydrolysates (PHS), seaweed extracts, chitosan, beneficial fungi and bacteria (du Jardin, 2015). Others may contain chitosans (a soluble version of chitin), inorganic compounds such as silicon. The primary sources of bio-stimulants also display various origins and physiological characteristics (Albrecht, 2019).

3. 3. 1 Humic substances (HS)

Humic substances are collections of natural components of the soil organic matter with relatively low molecular mass, resulting from the decomposition of plant, animal and microbial residues, and from the metabolic activities of soil microbes. Humic substances are often divided into fractions according to their molecular weight. The lower molecular weight fractions tend to have greater positive biological effects on plants (Halpern et al., 2015), Humic substances have many positive effects on the plant. They can improve soil physicochemical properties, root nutrient uptake and lateral root development. Humic substances have been recognized for as essential contributors to soil fertility because they have many effects on physical, physicochemical, chemical and biological properties of the soil. Most bio-stimulant effects of humic acid refer to the amelioration of root nutrition, through different mechanisms. One of them is increased uptake of macro and micronutrients due to the increased cation exchange capacity of the soil containing the polyanionic HS and to the increased availability of phosphorus by HS interfering with calcium phosphate precipitation (Calvo et al., 2014).

3. 3. 2 Seaweed extracts (SE)

Seaweed extracts are the new type of products currently used in crop production. These extracts are obtained from algae. They have been used as feed additives for animal nutrition improvement (Craigie, 2011) and used as industrial raw material or in the production of natural cosmetics. Seaweed extract is a heterogeneous substance that can be characterized by its parent material (Khan et al., 2009), the pH of the extraction solution, or H-NMR spectroscopy. Today, seaweed extracts obtained from marine algae are the subject of interest in agriculture with an emphasis on its application in sustainable agriculture. The most frequently used seaweeds in agriculture are known as the brown seaweeds, including species of the genera *Ascophyllum, Fucus,* and *Laminaria*. Most of the seaweed products are soluble powders or liquid formulations derived from different extraction procedures (Officer, 2014).

The biological activity of these extracts mainly depends on the raw material and the extraction process, which could be alkali extraction, acid extraction, or other technology (Battacharyya et al., 2015). Seaweed extracts perform as bio-stimulants mainly due to the presence of plant hormones. Mostly, auxins, cytokinins, gibberelins, abscisic acid and ethylene can be found. Auxins are needed for elongational growth of plant tissues and apical dominance, cell division, plant movements and plant aging. Cytokinins are important in cell division regulation affecting plant growth and rest period. Moreover, one of the basic functions of gibberellins is the initiation of seed germination, growth regulation, braking bud dormancy, florescence and fruits development. Abscisic acid and ethylene are capable for response to stress factors, inhibition of cell growth and acceleration of plant aging. Additionally, abscisic acid plays a major role in the regulation of seed germination (Officer, 2014).

3. 3. 3 Beneficial bacteria

Beneficial bacteria are also known as plant growth-promoting bacteria (PGPB) found in the bulk soil or rhizosphere and promote plant growth under some conditions. PGPB have diverse genera and abilities to promote plant growth in various different ways. PGPBs have been recognized to have a number of positive effects on plant growth. They can be used as pathogen control (Bashan, 2005) increased salt tolerance (Alavi et al., 2013), increased resistance to heavy metals and other toxins, increased growth and yield, and enhanced plant nutrition (Alam et al., 2011).

Plant-growth-promoting bacteria are found in the genera *Bacillus*, *Rhizobium*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, and many others. One of the well-known effects of PGPBs on plants is their ability to fix nitrogen. This ability is mostly associated with root-nodule-forming rhizobacteria, which live in a symbiotic relationship with leguminous plants. Another ability of PGPBs is their ability to produce siderophores, small iron-chelating compounds that reduce the growth of deleterious soilborne pathogens. PGPBs also can promote plant growth directly because they will produce plant hormones like auxins, cytokinins, and gibberellic acid, and indirectly by inducing hormonal changes within the plant host (Albrecht, 2019).

3. 3. 4 Beneficial fungi

Beneficial fungi with plant bio-stimulant activity are found in the group of symbiotic fungi, particularly arbuscular mycorrhizal fungi (AMF) within the genus *Glomus*, which penetrate plant roots and form a highly branched tree-like network of roots and hyphae. This network allows the plants to extend their root system beyond the depletion zone, allowing for improved uptake of nutrients and water and rendering them more tolerant to drought stress. Moreover, they can increase nutrient uptake. A good example of AMF is their improvement of phosphorous uptake, especially in phosphorous-deficient soils (Albrecht, 2019).

Some other plant-beneficial fungi are located within the genus *Trichoderma*, a group of hyphae-forming fungi found in the soil or on dead wood and bark. Trichoderma-based products have been particularly successful due to their capacity to control phytopathogenic fungi. They are no harm to humans, livestock and crop plants and in their natural environment colonize plant roots without any apparent adverse reactions. *Trichoderma* spp. are described as major plant growth-promoting fungi that widely exist in the natural environment. These *Trichoderma* strains can promote growth and reproduction and efficient transformation of soil nutrients. Moreover, they can change the plant rhizosphere soil environment and promote plant growth (Halifu et al., 2019).

3. 3. 5 Chitosan

Chitosan is a natural polysaccharide and biopolymer, is produced by alkaline deacetylation of chitin. Chitosan can also occur naturally in fungi and yeast. The chitosan has been interested in various fields due to its unique biological activities, such as biocompatibility, biodegradability, nontoxicity, antimicrobial activity, antitumor activity and immune-enhancing effect. Chitosan can be extracted from many things such as insects, yeast, mushroom and the cell wall of fungi, Moreover, it can also be extracted from marine shellfish such as crab, lobster, krill, cuttlefish, shrimp, and squid pens. Chitosan has been used in various applications such as agriculture, food processing, biotechnology, chemistry, cosmetics, dentistry, medicine, textiles, veterinary sciences, and environmental sciences. The polyelectrolyte nature and the presence of reactive functional groups are responsible for the gel-forming ability, high adsorption capacity, biodegradability, and antimicrobial properties of chitosan which in turn are essential for its commercial applications (Qavami et al., 2017). Chitosan is commercially produced from deacetylated chitin, which is the most abundant polysaccharide in nature following cellulose (Zou et al., 2016). Due to its remarkable physicochemical and biological properties, Chitosan is one of the most promising polymers for biomedical applications (Balan and Verestiuc, 2014). It is also used in artificial skin, hemostatic agents, and drug delivery systems.

3. 3. 6 Silicon (Si)

Silicon is a bio-stimulant in the group of inorganic products. It is the second-most abundant element in the Earth's crust following oxygen. Although most of Si is present in the form of insoluble oxides or silicates in the soil, some water-soluble Si also occurs. Si is a non-essential element for plant nutrition in the sense of classical criteria postulated by Arnon and Stout (Epstein, 1994). But, biostimulatory of Si effect on plant growth and development of higher plant have been well established (Ma and Yamaji, 2006). The advantageous properties of silicon are best documented regarding its positive effects on abiotic stress tolerance and resistance to pathogens and diseases. Silicon can be found as non-ionic silicic acid in the soil solution, which is easily taken up by plant roots and moved throughout the plant. It is accumulated at the endpoints of the transpiration stream in cell walls, cell lumens, and intercellular spaces in the form of hydrated amorphous silica (Savvas and Ntatsi, 2015). Highest concentrations are usually found around the stomata. These silica depositions or phytoliths increase leaf mechanical strength that lead to increase light interception and photosynthesis (Albrecht, 2019).

3. 4 Effect of biostimulant on crop response

The modern agricultural practices have been introducing towards organic, sustainable or environmentally friendly systems. These modern agricultural practices aim to reduce inputs without reducing the yield and quality. These goals can be achieved by breeding programs. However, these programs will take a long time to be completed. For vegetable crops, application of bio-stimulants can reduce chemical fertilizer input without affecting yield and quality. Biostimulants have the ability to improve the quality of leafy vegetables susceptible to nitrate accumulation such as rocket and can keep nitrates under limit imposed by EU regulations. For floriculture, bio-stimulants can promote the growth of plants, reach blooming and be commercial earlier (Bulgari et al., 2015).

3. 4. 1 Role of plant growth-promoting fungi (PGPF) in crop response

Generally, plant growth-promoting fungi considered to be beneficial for all plant species because of their conserved beneficial abilities. These beneficial abilities directly and indirectly influence the growth and productivity of a wide range of host plants. The reported beneficial effect of plant and PGPF interactions comprise the improvements in seed germination rate, seedling vigor, root development and morphogenesis, shoot growth, yield, photosynthetic efficiency, flowering, and plant composition. Plant growth promotion by PGPF may also variously arise from enhanced nutrient availability, amelioration of abiotic stresses, and antagonism to phytopathogens (Hossain et al., 2014).

Photosynthesis is the main source of carbon for green plants. Increased rate of photosynthetic potential may result in a higher rate of carbon assimilation in plants, which can be accelerated for faster development and higher biomass production. Many studies have also reported that PGPF can be used to enhance photosynthesis under suboptimal conditions. *Metarhizium anisopliae* LHL07 inoculated soybean plants significantly increased chlorophyll contents, transpiration rate, photosynthetic rate, and leaf area, under salt stress as compared to non-inoculated control plants (Khan et al., 2012). Root colonization with *T. atroviride* TaID20G improved the chlorophyll and carotenoid synthesis in maize seedlings, contributing to the alleviation of the drought stress (Guler et al., 2016). PGPF also increase the chlorophyll content and photosynthetic rate in the host plant under pathogen stress (Xia et al., 2016).

The positive effects of PGPF are found from the very the early stage of crop development influencing germination and seedling growth. Different types of PGPF species differ significantly in their effect on seed germination and seedling growth. Cucumber seeds were sown in soil amended with *T. harzianum* propagules gave a 30% increase in seedling emergence, 8 days after sowing (Yedidia et al., 2001). The rate of germination of maize and seedling growth of both maize and beans were increased by *Trichoderma*. The increased root length and collar diameter, stem length and diameter by *Trichoderma* treatment are measures of seedling's survivability and illustrate the direct effect of the fungus on the plants (Okoth et al., 2011). Uptake of nutrients by the plant is the main function of plant roots to support growth and development of the plant. The plant root system is in closest contact with soil microbial populations; therefore, the root system functions under the direct influence of microbial interaction. Many studies have shown that PGPF can significantly enhance the root growth. Plants inoculated with some PGPF had greater root biomass of the root system than the control plants (Hossain et al., 2014).

3. 4. 2 Role of plant growth promoting bacteria (PGPB) in crop response

Plant growth-promoting bacteria (PGPB) have many benefits in agriculture by increasing crop productivity and nutrient content and suppressing the growth of pathogens. Interaction of beneficial plant-microbe based on genomics, transcriptomics, proteomics and metabolomic data of PGPB. The host plant will lead to enhance microbial inoculants for increasing crop yield and nutrient content. PGPBs are described as a green technology due to reducing the use of chemical fertilizers thereby improving soil health (Ramakrishna et al., 2019). In the global market, the use of biostimulants in agriculture increase per year. Approximately 12% has been reported (Calvo et al., 2014). Large-scale commercial production has been achieved with some PGPB like *Burkholderia*, *Pseudomonas, Rhizobium, Azospirillum, Azotobacter, Bacillus and Serratia sp*. (Parray et al., 2016).

To the main important groups of PGPB belong Proteobacteria and Firmicutes (Khalil 2016). *Bacillus sp*. are the predominant bacteria with plant growth-promoting activity in the phylum *Firmicutes*. In the phylum *Proteobacteria*, class Gamma proteobacteria involve the genera *Pseudomonas*, *Acinetobacter*, *Serratia*, *Pantoea*, *Psychrobacter*, *Enterobacter* and *Rahnella*. *Fabaceae* (legume family) contains important agricultural plants such as soybean (*Glycine max*), pea (*Pisum sativum*) and alfalfa (*Medicago sativa*) are the host plants that can be associated with PGPB. In this family, the symbiotic relationship between nitrogen-fixing endophytic bacteria and leguminous plants has been well characterized (Oldroyd et al., 2011). For phytoremediation of metal contaminated soil, maize, sorghum and barley belonging to *Poaceae* family have been used because of their high biomass and potential use for biofuels (Vamerali et al., 2010). The PGPB associated with these plants are related to free-living

Pseudomonas sp. and *Burkholderia sp*. as well as endophytic *Bacillus sp.* interacting with hyperaccumulator plants. *Brassica juncea and Brassica napus.*

PGPBs also can promote plant growth directly because they will produce plant hormones like auxins, cytokinins, and gibberellic acid, and indirectly by inducing hormonal changes within the plant host (Albrecht, 2019). The term plant growth-promoting bacteria belong to bacteria that colonize the rhizosphere and promote plant growth. These bacteria can play an essential role in helping plants to establish and grow in nutrient-deficient conditions. PGPBs helps plants directly or indirectly to increase plant growth-promoting attributes such as increase in seedling emergence, effective nodulation as well as nodule functioning, enhanced, increased indigenous plant hormones, root hair proliferation, root hair deformation and branching, early mineral and water uptake, promote the accumulation of carbohydrates and increasing the yield (Kishore, 2006)

Leguminous plants such as soybean, pea, peanut, and alfalfa can be established symbiosis forming nodules on roots of plants by atmospheric N-fixing bacteria such as *Rhizobium* and *Bradyrhizobium*, which convert nitrogen into ammonia which is used as a source of nitrogen by plants (Murray, 2011). Free-living bacteria such as *Azospirillum, Azoarcus, Azotobacter, Bacillus polymyxa*, *Burkholderia*, *Gluconoacetobacter* and *Herbaspirillum* have the ability to fix nitrogen. These PGPB can be used in several important crop production such as wheat, sorghum, maize, rice and sugarcane (Pérez-Montaño et al., 2014).

3. 4. 3 Role of seaweed extracts on crop response

Seaweed and seaweed-derived products have been widely used as biostimulants in agricultural production as the presence of multiple growth regulators such as cytokinin, auxins, gibberellins, betaines. Moreover, they contain a considerable amount of macronutrients such as Ca, K, P, and micronutrients like Fe, Cu, Zn, B, Mn, Co and Mo, which are required for crop growth and development. Many studies have reported that there is a wide range of beneficial effects of seaweed extract on crops. These beneficial effects are seed germination and establishment, enhance crop performance and yield, inducing resistance to biotic and abiotic stress (Begum et al., 2018).

Today, seaweed extracts are used in agricultural production as commercial products such as seaweed liquid fertilizers (SLF) are available as manure, foliar spray, soil conditioners and soil drench (Thirumaran, 2009). Different formulations of seaweed are available in the market such as SLF, granular and powder. Chemical fertilizers were found to be less effective compared to seaweed fertilizer because of the high level of organic matter aids in retaining moisture and minerals in upper soil that is available to roots (Sivasankari et al., 2006)

The increase in germination percentage and seedling vigor in case of green gram (*Vigna radiata*) (Venkataraman and Mohan, 1997) and cowpea (*Vigna sinensis*) (Sivasankari et al.2006) was also described. The higher germination percentage and seedling vigour at low concentrations of seaweed extracts might be due to the presence of growth promoting substances such as auxins, gibberellins, phenyl acetic acid (Sivasankari et al., 2006) and other micro-nutrients (Layek et al., 2014). It has been also reported that application of seaweed both improved the growth of the crop and helped in increasing the number of functional nodules as compared to control due to presence of several cytokinins, which are found in brown algale extracts, including, trans-zeatin riboside, and its dihydro derivatives (Saravanan et al., 2003).

The seaweed extracts increased the growth parameters, yield attributes of maize and its application gave the maximum return. Moreover, presence of some macro and microelements and plant growth regulators, especially cytokinins, indole acetic acid and gibberellic acid, GA in Kappaphycus and Gracilaria extracts are responsible for the increased yield (Pal et al., 2015).

3. 4. 4 Role of humic substances on crop response

Humic substances (HS) have been used in crop production. They are widely known as a plant growth promoter mainly by changes on root architecture and growth dynamics, which result in increased root size, branching and/or greater density of root hair with larger surface area. The effect of humic substances application is significantly strong during germination and initial plant growth. Some studies showed that there was the favourable effect of humic substances on development of roots of maize seedling, activity of plasma membrane H+ATPase, carbohydrates and N metabolism and photosynthesis. Humic substances application in field crops and vegetables stimulate the root system development and yield (Szczepanek and Wilczewski, 2016). In maize cultivation, a positive effect of humic substances on the whole plant growth had been reported, including roots, stems and leaves (Eyheraguibel et al.,2008).

3. 4. 5 Role of chitosans on crop response

Application of new technologies have resulted in rapid advances in agriculture and made it possible to achieve target of crop production. However, for sustainable agricultural production, more environmentally friendly production technologies must be followed. Chitin, a homo polymer comprising b-(1-4)-linked N-acetyl-D-glucosamine residues, is one of the most abundant, easily obtainable and renewable natural polymers, second after cellulose (Katiyar, 2015). Chitosan has been widely applied in functional foods, food additives, environmental protection and biotechnology (Shahidi et al., 1999). Moreover, various studies have shown that chitosan has antifungal and antimicrobial effects (Kumar et al., 2004). The positive effect of physiological and biological properties of chitosan have led to its use in various industries, including agriculture, as a coating material for fruits, seeds and vegetables (Lee et al., 2005). Chitosan promotes plant immune systems and protects plants against attack by micro-organisms. Moreover, it can also improve growth and crop productivity.

Chitosan has been described as ''plant defense booster". The term plant defense booster applies to a group of compounds, which act by triggering various physiological and morphological responses within the plant that help to stimulate natural defense mechanisms. The importance of plant defense boosters is that they can help to reduce the amount of chemicals applied to crops in crop protection. Chitosan is one of the most important elicitors. Researchers have shown that it elicit plant defense responses to a broad spectrum of phytopathogens, including plant virus (Terry and Joyce, 2004). Bean with chitosan treatment decreased the number of local necroses caused by alfalfa mosaic virus (AMV) infection. It has been reported that chitosan inhibited the infection caused by the bacteriophage, the efficiency of inhibition of bacteriophage infection depends directly on the final concentration in the medium (Ma et al., 2008).

Ma et al., 2014 reported that oligo-chitosan stimulated wheat growth in terms of germination capacity, root length, seedling height and increase in root activity. Guan et al., 2009 also showed that seed soaked with chitosan increased the germination percentage of maize seed. Another study had been reported that seed priming with chitosan enhanced seed germination and seedling vigor in pearl millet (Duke and Powles, 2008).

The leaf water content reflects the water status of the plant. Chitosan coating can increase the leaf water content of seedlings. The results from one study showed that chitosan significantly increased the concentration of chlorophyll under drought stress, which illustrates that chitosan can enhance the photosynthesis performance and the accumulation of organic matter in wheat seedlings. A well-developed root system absorbs more water to keep the moisture stable under the drought condition. Under drought stress condition, chitosan coating can reduce the inhibition of roots and stem grow, which show the chitosan can effectively promote the development of root system and strengthens the capability of water absorption, so as to enhance drought resistance of wheat seedlings (Zeng and Luo, 2012).

Chitosan can be used as a coating material for fruit (Jiang and Li, 2001). Some other previous studies showed that chitosan coating has the potential to prolong storage life and to control the decay of many fruits such as strawberries papaya (Sivakumar et al., 2005). The chitosan-based coating material can be used as a protective barrier on the surface of fresh fruit, reduce water loss, inhibit gas exchange, decrease nutrient loss, and prevent fruit rotting responsible microorganisms growth that causes fruit rotting (Qiuping and Wenshui, 2007). The combined application of chitosan and ammonium carbonate offers a commercially acceptable, economically viable and effective alternative for postharvest control of anthracnose in stored papaya. Another study had been documented that dipping papaya in chitosan plus ammonium carbonate, significantly ($P < 0.005$) retarded color development of skin and flesh. Moreover, it can increase fruit firmness and reduced weight loss. Many studies have been documented that the effectiveness of chitosan depended on molecular weight, the ratio of sugar carbons to glucosamine and N-acetyl-glucosamine, and the concentration and frequency of applications (Katiyar et al., 2015).

3. 4. 6 Role of silicon on crop response

Silicon (Si) is classified as a beneficial element. The use of silicon in agriculture is most common in the production of vegetables in greenhouses. But the use of silicon for the fertilization of agricultural plants is rare. Positive effects of silicon fertilization are associated with foliar application because it is much cheaper and more convenient to use than soil fertilization. Silicon foliar application has a biostimulative effect. The better results were observed in stressful conditions for plants such as salinity, deficiency or excess of water, high and low temperature, and the strong pressure of diseases and pests, etc (Artyszak, 2018).

There was an evaluation of the silicon foliar application effect of on wheat production. This research was conducted in Iran. In this study, foliar application of 6 mM sodium silicate at various stages of wheat growth provided higher resistance to drought by maintaining cellular membrane integrity, relative water content, and increasing chlorophyll content. The best positive influence of silicon application was observed in the use of silicon both at the tillering and anthesis stages (Maghsoudi et al., 2016).

Other studies conducted in India, foliar application of silicic acid (2% soluble H_4SiO_4) at a dose of 2 or 4 $cm^3 \cdot dm^{-3}$ doubled or tripled, and thus significantly improved, soybean growth and yield (Shwethakumari and Prakash, 2018). For economic reasons, the authors recommended using a smaller dose (2 $cm^3 \cdot dm^{-3}$) in three sprays. Growth and yield attributes of maize hybrids like plant height, stem diameter, leaf number, cob length, number of grains per cob, 100 grain weight, grain yield and biological yield were found to be adversely affected by drought in the present study. However, silicon application significantly improved these plant attributes (Amin et al., 2018).

3. 5 Effect of biostimulant on nutrient uptake

The application of chemical fertilizers in crop production is inexpensive and effective method of supplying crops with mineral nutrients (Chen, 2006). To reduce the chemical fertilizers, organic fertilizers can be used in agricultural production, such as compost, sludge, or manure. They have advantage in increasing the efficiency of nutrients already available in the agro-ecosystem, and they require little input of energy to be processed. Moreover, the mineral nutrients that are bound in organic materials may be more stable. Therefore, they may not be washed away from the field or released into the atmosphere easily (Estavillo et al., 1994) and (King and Torbert, 2007). However, organic fertilizers have some problems with supplying crops with nutrients water-soluble form when the crops need them (Chen, 2006). There is one possible way to overcome this disadvantage that crops have to be grown with more robust root systems and higher nutrient-uptake efficiency, to ensure that they receive the nutrients when they need them. There is also another way to make nutrients more available form by promoting certain types of organisms within the soil microbial community (Vessey, 2003). These approaches can be achieved by introducing bio-stimulants to crop leaves, seeds, or soil as a means of stimulating root growth.

3. 5. 1 Role of plant growth promoting bacteria on nutrient uptake

Symbiotic N_2 fixation is one of the important biological processes. It is very important for the development of sustainable agriculture. This process converts the atmospheric N_2 to ammonia with the aid of a key enzyme called nitrogenase. This symbiotic process is achieved by bacteria inside the cells of *de novo* formed organs, the nodules, which usually develop on roots of various leguminous plants. This process is result of a complex interaction between the host plant and rhizobia (*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium* and *Mesorhizobium*). This symbiotic relationship is beneficial for both symbiotic partners because the host plant provides the rhizobia with carbon and source of energy for growth and functions while the rhizobia fix atmospheric N_2 and provide the plant with a source of reduced nitrogen in the form of ammonium (Sulieman and Tran, 2014). Therefore, this process is an economically attractive practice because we can reduce external inputs and improve internal resources. One study showed that combined inoculations of endophytic diazotrophic bacteria such as *Gluconaceto-bacter diazotrophicus,Burkholderia tropica, Azospirillum amazonense, Herbaspirillum rubrisubalbicans,* and *Herbaspirillum seropedicae* were very effective at promoting N fixation in sugar cane (Oliveira et al., 2009).

Phosphorus can be found in agricultural soil. P in agricultural soils is present in the forms of inorganic and organic. The insoluble inorganic forms account for about 20–50% of the total soil P, usually in the form of PO_4-ions. These ions can be adsorbed onto the positively charged constituents of the soil, or they form poorly soluble precipitates with Fe, Al, or Ca, depending on the pH (Halpern et al., 2015). When soluble inorganic phosphate applied to soil as chemical fertilizer, it is rapidly immobilized after application and becomes unavailable to the plants. Bacteria try usually a number of strategies to solubilize the insoluble inorganic and organic P compounds. Generally, this P fixation and precipitation of P in soil is highly dependent on pH and soil type. Therefore, phosphorus is fixed by free oxides and hydroxides of aluminum and iron in acid soils. Moreover, in alkaline soils it is fixed by calcium, causing a low efficiency of soluble P fertilizers, such as apatite based rock phosphates (Rodríguez and Fraga, 1999). To solubilize inorganic P, bacteria have ability to synthesis organic acids such as gluconic and citric acids, which chelate the insoluble compounds and lower the pH that leads to increase P solubility (Gamalero and Glick, 2011). Another mechanism is to simply release protons, which lowers the pH and increases solubility without the help of chelates (Gamalero and Glick, 2011). The ability to solubilize P is common in rhizosphere bacteria (Halpern et al., 2015), and many such bacteria have been isolated, including those from the genera *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Enterobacter, Streptomyces, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobactrium, and Erwinia* (Gamalero and Glick, 2011). Furthermore, several research papers of simultaneous growth promotion and increase in P uptake by plants as the result of phosphate-solubilizing bacteria inoculations have been described. Inoculation with two strains of *Rhizobium leguminosarum* selected for their P-solubilization ability has been reported that they can improve root colonization and growth promotion and significantly increase the P concentration in lettuce and maize (Halpern et al., 2015).

Fe is also abundant in soils but mostly in the insoluble Fe^{3+} oxide form, such as hematite, goethite, and ferrihydrite. In calcareous soils, Fe is mostly unavailable for plant uptake because the alkaline conditions render the Fe less soluble. Certain bacteria produce siderophores, which chelate Fe, making it more soluble. One study showed that maize and sunflower have better Fe uptake in nonsterile calcareous soils than in their sterile counterparts (Masalha et al., 2000). Similarly, another study showed that Pseudomonas ssp. increases Fe uptake and reduces chlorosis in mung bean (Sharma et al., 2003).

3. 5. 2 Role of plant growth promoting fungi on nutrient uptake

Biofertilizers are a mixture of naturally occurring substances. They are used to improve soil fertility in crop production. These fertilizers are very useful for not only soil health but also plant growth and development (Sadhana, 2014). Arbuscular mycorrhizal symbiosis contributes to the sustainability of the soil-plant system. The symbiotic relationship between AM and the roots of higher plants support effectively to plant nutrition and growth and has been shown to increase the productivity of many crops including maize (Augé, 2001). Many studies have shown that abuscular microrrhizal fungi (AMF) have positive effects on soil health and crop productivity. Thus, in the near future, AMF could be used as a replacement of inorganic fertilizers because mycorrhizal application can effectively reduce the amount of use of chemical fertilizer input especially phosphorus (Ortas, 2012).

P is one of the most important for plant growth and makes up about 0.2% of dry weight. However, it is one of the most difficult macronutrients for plants to acquire from the soil. In soil, it can be found in relatively large amounts. But, it is very limited available for plants because of the very low solubility of iron-, aluminum- and calcium- phosphates. According to many studies, AMF can possibly lower down the use of chemical fertilizers up to 50% for the best agricultural production. However, it may depend on the type of plant species and the prevalent stressful regimes (Smith et al., 2011).

AMF are soil-borne fungi. They can effectively improve plant nutrient uptake and resistance to several abiotic stress factors (Sun, 2018). The main species of AMF belong to the sub-phylum *Glomeromycotina*, of the phylum *Mucoromycota* (Spatafora et al., 2016). There are four orders of AMF, namely, *Glomerales, Archaeosporales*, *Paraglomerales*, and *Diversisporales*. They have been identified in this sub-phylum that also includes 25 genera (Redecker et al., 2013). Many studies showed that AMF colonization can be used to stimulate nutrient uptake in plants. Inoculation of AMF can increase the concentration of various macro-nutrients and micronutrients significantly (Chen et al., 2017). AMF have the ability to stimulate the uptake of inorganic nutrients in almost all plants, especially phosphate (Orientador, 2014). Even under deficient soil condition, AMF are also very effective in helping plants to take up nutrients from the nutrient-deficient soils (Kayama and Yamanaka, 2014). AMF can grow symbiosis with roots to obtain essential nutrients from the host plant. Then, they provide mineral nutrients such as N, P, K, Ca, Zn, and S in return. AMF usually can produce fungal structures like arbuscules. These structures are very helpful in the exchange of inorganic minerals and the compounds of carbon and phosphorus, ultimately imparting a considerable vigor to host plants (Maček, 2017; Kahlon and Malhotra, 1986). Therefore, the use of AMF can significantly increase the phosphorus concentration in both root and shoot systems (G and A, 2017). The mycorrhizal association can increase phosphorus supply to the infected roots of host plants under phosphorus-limited soil conditions (Bucher, 2007). In maize and other crops, the most widely recognized contribution of AM fungi to host-plant nutrition involves their ability to extract P from outside the P depletion zone around plant roots (Liu et al., 2000).

3. 5. 3 Effect of humic substance on nutrient uptake

Humic substance (HS) can be found in soil that stimulates the growth of root and shoot by improving mineral nutrition beneath the soil surface. The activity of humic substances in the soil can be evaluated in terms of the yield and active growth of plants (Zandonadi et al., 2016). Plant growth and mineral assimilation can be regulated by HS through their complementary and potentially diverse effects. Generally, these effects are recognized as direct and indirect (Zandonadi et al., 2013). Structural features, functional groups, and their tendency to interact with inorganic and organic ions and molecules residing in the soil substrate influence on activities of HS (García-Mina et al., 2004). Furthermore, under nutrient deficient soil, HS strongly influence on nutrient bioavailability via their ability to form complexes with metallic ions, which improves the availability of micronutrients (zinc, manganese, copper, and iron) and macronutrients (phosphorus) (García et al., 2016).

Humic substances have a variety of positive effects in crop production because they increased root dry weight and enhanced micronutrients such as Zn^{2+},Fe^{3+}, Mn^{2+}, and Cu^{2+} (Sharif et al. 2002). Atiyeh et al., 2002 showed that HS increased root dry weight of tomato and cucumber and stimulated root development and enhanced amounts of N, K^{+}, Cu^{2+}, and Mn^{2+} in ryegrass (Bidegain et al., 2000). They also increased root fresh and dry weights in tomato and eggplant (Dursun et al., 1999). Another study reported that humic acid affected fresh and dry weights of tomato roots and resulted in conspicious increase of Fe^{3+} content, depending on the humic resource (Adani et al., 1998).

3. 5. 4 Effect of seaweed extract (SE) on nutrient uptake

For many years, seaweed has been applied as a fertilizer in coastal regions (Craigie, 2011). Some plant nutrients can be found in SE. When applied to plants grown in a nutrient-deficient medium soil, the simple availability of these nutrients may improve growth and nutrient uptake. Usually, SE is produced from a brown seaweed, *Ascophyllum nodosum* that is commonly found in the North Atlantic, although other species, such as *Durvillaea antarctica*, *Durvillaea potatorum*, *Macrocystis pyrifera*, and *Ecklonia maxima* are also used (Khan et al., 2009).

The positive effect of seaweed extract application is as a result of many components that may work synergistically at different concentrations, (Fornes et al., 2002). In recent years, the use of seaweed extracts in agriculture was popular due to their potential use in organic farming and sustainable agricultural system (Russo and Berlyn, 1991), especially in rainfed crops. People want to avoid excessive fertilizer applications and to improve mineral absorption. Although chemical fertilizers have many negative effects to the environment and humans, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Kahlon and Malhotra, 1986).

One study showed that foliar application of SE obtained from the red alga *Kappaphycus alvarezii* increased the grain concentration of N, P, K, and S by up to 36%, 61%, 49% and 93%, respectively in soybeans grown under rainfed conditions (Rathore et al., 2009). Another study reported that the effects of foliar application of three different commercial SE on nutrient uptake in a 1-year-old grapevine planted in perlite medium. The vines were grown with a supply of mineral nutrients at optimal or high levels; result in all three SE induced significant improvement in macronutrient and micronutrient concentrations in the leaves. However, there was no effect observed when plants were grown at suboptimal mineral nutrition (Turan and Köse, 2004). (Crouch et al., 1990) measured the effects of a commercial SE on growth, as well as nutrient uptake such as Ca, Mg, and K. In this study, the growth of nutrient- stressed lettuce was not improved by root flushing with SE. However, nutrient uptake and plant growth were positively affected when the SE was applied to lettuce plants that were also receiving a highly concentrated nutrient solution.

3. 5. 5 Effect of chitosan on nutrient uptake

Environmental friendly agricultural practice is at a critical point due to the high-level production and usage of inorganic fertilizers. Thus, biodegradable biofertilizers, chitosan, are one of the popular methods within the research community to avoid the hazards of using inorganic fertilizers. Chitosan gets degraded enzymatically without interrupting the soil-borne beneficial rhizosphere biota at low concentrations. And, it can also induce the symbiotic exchange between plant and microbes (Escudero et al., 2017). In addition, as chitosan is a polysaccharide-based biopolymer, it stimulates the activity of plant symbiotic microbes, resulting in the alteration of rhizosphere microbial equilibrium, (Murphy et al., 2000).

Both, chitin and all its derivatives, have a high nitrogen content of 6.1% - 8.3%. Chitin has a high thermal and chemical stability, making it possible to store dry product for a good length of time. In addition, it can quickly be utilized as both a nitrogen source and an energy source by plants and microbes when added to crops. Plants can obtain the nitrogen in chitin from microbial breakdown and the release of inorganic nitrogen (Yen and Mau, 2007).

Late blight is an important disease in potato cultivation that causes economic damage to potato yields. However, one study had been recorded that after soil inoculation with chitosan as a biofertilizer, a significant reduction in tuber infestation by late blight was detected and also a significant increase in plant nutrient uptake was also recorded (O'Herlihy et al., 2003). In addition, 1% chitosan combined with fertilizer improved the nitrogen and phosphorous content in the roots and shoots of *Eustoma grandiflorum* compared with non-chitosan mixed soil grown plants (Ohta et al., 2002). Another observation was found in Chinese cabbage, plants treated with a chitin-based product showed faster growth than plants treated with a standard mineral fertilizer (Spiegel et al., 1988).

CHAPTER 4

4. MATERIAL AND METHODS

4. 1 Experiment Site

Screening pot experiment was established in soil from Kunvald-Bubnov (50°08'39.27'' N; 16°30'53.21'' E), which is typical with low content of P. The content of P and other available nutrients as well as trace elements determined in Mehlich 3 extracts (Mehlich, 1984) are given in Table 4-1 (a-c). Soil pH (5.20), as well as mineral nitrogen content was determined in 0.01 mol/l $CaCl_2$ after Houba et al., (1986) and pH after ISO 10390 (1994), respectively).

Table 4-1 The contents of available elements in soil determined in Mehlich 3 extract (in mg/kg)

(a) The content of available macronutrients

N_{min}*	P	K	Ca	Mg	S
9.52 (±0.41)	22 (±0.12)	73 (±0.57)	2231 (±4.64)	181 (±0.19)	10 (±0.35)

* mineral nitrogen was determined with 0.01 mol/l $CaCl_2$ extraction.

(b) The content of available micronutrients

Fe	Cu	Zn	Mn	B	Mo	Ni
181 (±2.12)	2.06 (±0.03)	3.65 (±0.03)	74 (±1.17)	<0.03	<0.01	<0.005

(c) The content of available form of other elements

Al	Na	Pb	Cd	As	Cr
1021 (±4.52)	53 (±0.46)	<0.02	<0.001	<0.03	0.03 (±0.00)

Pot experiment with maize (not treated seeds of variety Colisée) was established in the climate chamber in the greenhouse of Czech University of Life Sciences Prague on 22th April 2015. Temperature was set up to 25°C and humidity at 70 %. Into the 500 ml pots was weight 538 g of above mentioned 1soil sieved through 2 mm mesh. This corresponds to 500 g of dry soil. Rock phosphate powder fertilizer was thoroughly mixed with soil. The dose of the fertilizer was applied to reach the level 24 mg P per kg of soil. Furthermore, mineral nitrogen (ammonium nitrate) was applied to obtain the same value 1 g N per kg of soil based on the fertilizer's analysis. Total nitrogen and hosphorus contents in fertilizers and their doses are given in Table 4- 2.

Table 4- 2 The amount of fertilizer applied to the screening pot experiment

Fertilizer	P (%)	N (%)	Added P (mg/pot)	added N (mg/pot)
Rock phosphate	8.00	34.0	12	50

Four maize seeds were sown per pot. Biostimulants were applied immediately after sowing in the doses recommended by producers (Table 4-3, (a and b)). They were always mixed with chlorine free tap water to prepare stock solution and subsequently applied with pipette on the soil surface (10 or 20 ml of stock solution per pot).

Table 4- 3 (a) A Kind of biostimulants used in the pot experiment and their active substances

Biostimulant	Active substance	Strain	Producer
Proradix	Pseudomonas	DSMZ 113134	Sourcon Padena, Germany
Rhizovital 42	Bacillus amyloliquefaciens	FZB 42	ABiTEP, Germany
Rhizovital 45	Bacillus Amyloliquefaciens	FZB 45	ABiTEP, Germany
MUCI	Paenibacillus mucilaginosus	JX-1	ABiTEP, Germany
SuperFifty	AE* Ascophyllum nodosum	-	Agriges, Italy
NemaTec	AE Laminaria sp.**	-	Agriges, Italy
LamVita	AE Laminaria Sp.	-	Agriges, Italy
Biological fertilizer OD	Penicillium bilalii	OD	Bayer Crop Sci., Germany
Trichoderma OMG	Trichoderma harzianum	OMG08	Anahlt University, Germany

* AE – Algae extract, ** extracted laminarin only

Table 4- 3 (b) Biostimulants' dosage and volume of stock solution per pot

Biostimulant	Dose per pot	Volume of stock solution per pot
Proradix	13.5 µg	20 ml
Rhizovital 42	35.0 µl	20 ml
Rhizovital 45	35.0 µl	20 ml
MUCI	5.0 ml	20 ml
SuperFifty	16.0 µl	10 ml
NemaTec	40.0 µl	10 ml
LamVita	40.0 µl	10 ml
Biological fertilizer OD	0.5 ml	20 ml
Trichoderma OMG	65.0 µl	20 ml

Immediately after biostimulants application, all pots were watered on 60 % of water holding capacity (WHC) to improve their distribution into the soil. This WHC was maintained during the whole time of the experiment. Pots were randomized once per week. After two weeks of growing, maize plants were selected to final number of 2 plants per pot.

4. 2 Evaluated parameters and analysis

4. 2. 1 Basic parameters

Plant height was measured two times during the experiment – second and fifth week after sowing. The final harvest was realized 7 weeks after sowing. Harvested aboveground biomass of plants was weight and air-dried for analysis. The weight of air-dried plants is further described as dry mass yield.

4. 2. 2 Plant analysis

Dried aboveground biomass of plants was fine milled and analyzed as following: total contents of elements in the plant samples were determined in mineral extracts obtained by dry decomposition (Mader et al., 1998). Samples were decomposed first on a hot plate and then in a muffle furnace with a stepwise increase of the ashing temperature to 500 °C. The ash was dissolved in 1.5% HNO_3 solution. The contents of studied macro- and miocroelements were determined using optical emission spectrometry with inductively coupled plasma – ICP-OES (Varian Vista Pro, Australia).

4. 2. 3 Data analysis

In this experiment, Microsoft Office Excel was used to interpret recorded data and the result data were computed statistically by using STATISTIX program (Version 8). Mean comparisons were analyzed with the use of Least Significant Difference (LSD) test at 5 % level.

CHAPTER 5

5. RESULT

Maize (Variety Colisée) was grown in a greenhouse under treatments consisting of two types of fertilizer application, biostimulants only and combined application of rock phosphate (RP) with biostimulants. Proradix, RhizoVital 42 (RV42), RhizoVital 45, MUCI, RV42 + MUCI, SuperFifty, NemaTec, LamVita, Biological Fertilizer OD and Trichoderma OMG were used as biostimulants to investigate the effect on maize growth in both applications. Only bostimulants application includes a control (soil only), Proradix, RhizoVital 42 (RV42), RhizoVital 45, MUCI, RV42 + MUCI, SuperFifty, NemaTec, LamVita, Biological Fertilizer OD and Trichoderma OMG treatments. Rock phosphate application includes control (soil + RP), Proradix + RP, RV42 + RP, RhizoVital 45 + RP, MUCI + RP, RV42 + MUCI + RP, SuperFifty + RP, NemaTec + RP, LamVita + RP, Biological Fertilizer OD + RP and Trichoderma OMG + RP treatments, respectively. In our experiment, plant height and aboveground dry biomass weight were measured to investigate the relation between biostimulants and maize growth. Besides, macro and micronutrients content in aboveground biomass, and macro and micronutrients uptake in aboveground biomass were also analyzed.

5. 1 Biostimulants treatments

5. 1. 1 Plant height

Plant height was measured two times, two weeks after sowing and five weeks after sowing. The effect of biostimulants on plant height and above biomass dry weight is shown in (Figure 5- 1 and Figure 5- 2). According to our results, the highest growth was recorded for Proradix (24.1 cm) followed by Biological fertilizer (23.9 cm), and RhizoVital 45 (22.9 cm) at two weeks after sowing. However, the application of Muci (18.4 cm) and RV42 + MUCI (20.1 cm) resulted in plant height decrease. At five weeks after sowing, the highest plant growth was found in NemaTec (71.0 cm), whereas the lowest plant height was found again at MUCI (62.8 cm) and RV42 + MUCI (64.4 cm).

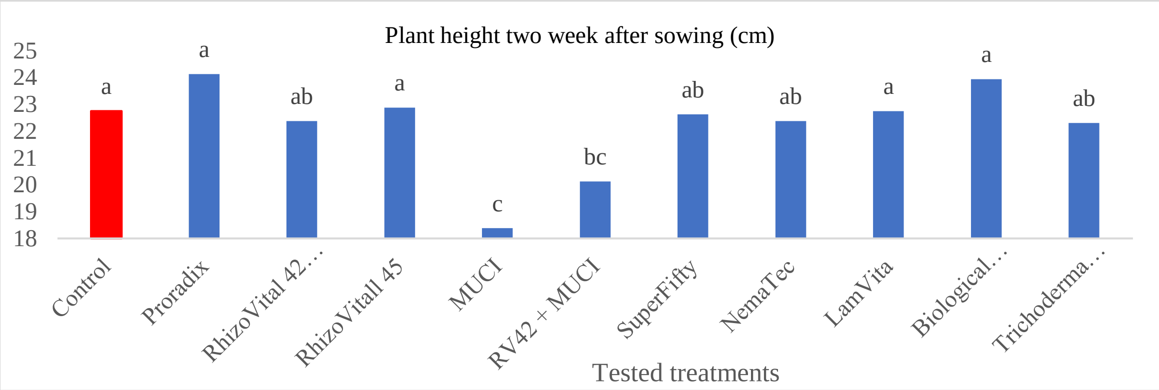

Figure 5- 1 Plant height affected by biostimulants application

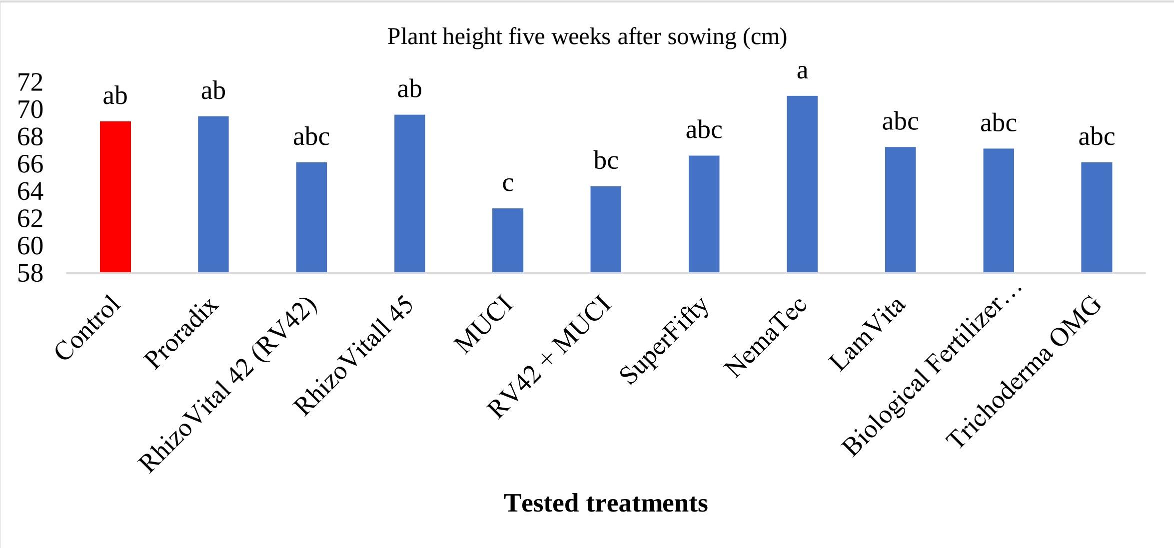

Figure 5- 2 – Plant height affected by bio-stimulants application

5. 1. 2 Aboveground biomass dry weight

After harvest, the aboveground biomass of plants was air-dried and weight for analysis (Figure 5- 3). Different treatment significantly ($p < 0.05$) influenced aboveground biomass dry weight. Proradix (1.88 g) increased aboveground biomass dry weight significantly, followed by biological fertilizer and control (soil only). The minimum was found for Muci (1.38 g) and RV 42 + MUCI (1.43 g).

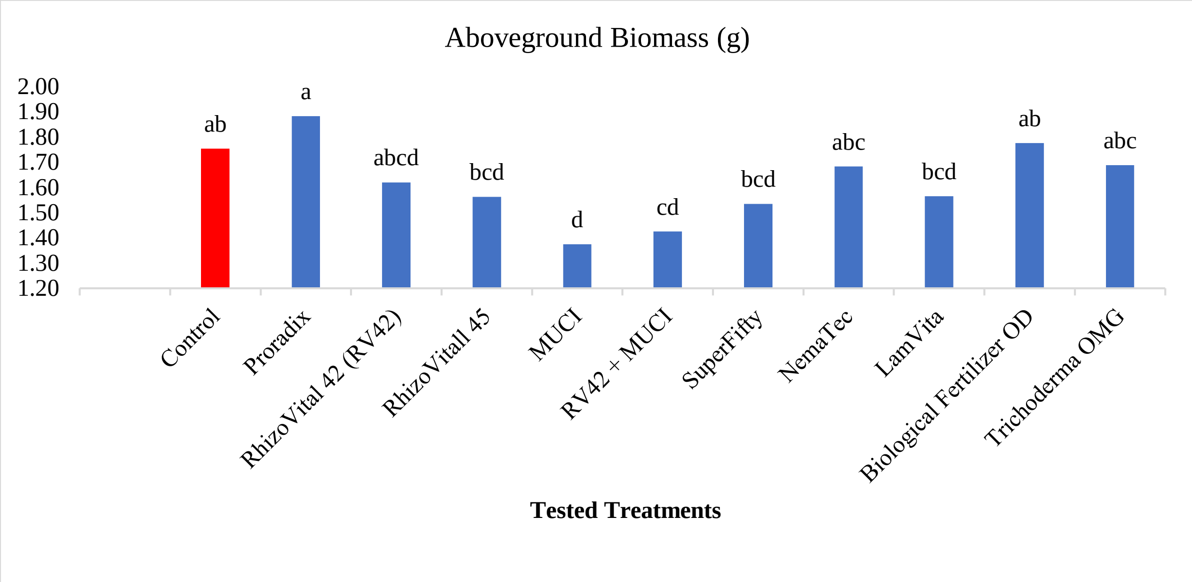

Figure 5- 3 Above biomass affected by biostimulants application

5. 1. 3 Macronutrients content in aboveground biomass

P, K, Ca, Mg, and S content in aboveground biomass were analyzed in our experiment (Table 5- 1). In this experiment, the highest P content in aboveground biomass was found at Trichoderma OMG (1795 mg/Kg) followed by control (1767 mg/kg) and RhizoVital 42 (1701 mg/kg), while the lowest content was found at RV42 + MUCI (1447 mg/kg). The maximum amount of K content was observed by Trichoderma OMG (13304 mg/kg). However, the highest content also significantly occurred in control (12173 mg/kg) and RhizoVital 42 (12108 mg/kg) treatments, respectively. Application of MUCI (10630 mg/kg) and RV42 + MUCI (9703 mg/kg) also resulted in the low content of K. For Ca content, RhizoVital 45 resulted the highest content (8536 mg/kg) among treatments although the lowest amount was recorded with Proradix (6704 mg/kg). Big differences were found among Mg contents. The highest Mg content was found for RhizoVital 45 (3395 mg/kg). However, the lowest content was obtained at Proradix (2625 mg/kg). The S content was significantly different within treatments. The highest content was found in LamVita treatment (677 mg/kg) while the lowest content was recorded at Biological Fertilizer OD (453 mg/kg).

Table 5- 1 Effect of biostimulants on macronutrients content in aboveground dry biomass

Treatment	P(mg/kg)	K(mg/kg)	Ca(mg/kg)	Mg(mg/kg)	S(mg/kg)
Control	1767 ^{a}	12173 ^{ab}	8276 ^{ab}	3175 ^{ab}	570 ^{abcde}
Proradix	1579 ^{ab}	11910 ^{ab}	**6704 ^{d}**	**2625 ^{c}**	505 ^{cde}
RhizoVital 42 (RV42)	1701 ^{ab}	12108 ^{ab}	7349 ^{bcd}	2953 ^{bc}	557 ^{bcde}
RhizoVital 45	1689 ^{ab}	11730 ^{ab}	8536 ^{a}	3395 ^{a}	593 ^{abcd}
MUCI	1682 ^{ab}	**10630 ^{b}**	7496 ^{bcd}	3103 ^{ab}	**490 ^{de}**
RV42 + MUCI	**1447 ^{b}**	**9703 ^{b}**	7896 ^{abc}	2903 ^{bc}	559 ^{bcde}
SuperFifty	1651 ^{ab}	11603 ^{ab}	8205 ^{abc}	3155 ^{ab}	659 ^{ab}
NemaTec	1536 ^{ab}	10650 ^{b}	7793 ^{abc}	3138 ^{ab}	614 ^{abc}
LamVita	1688 ^{ab}	11490 ^{ab}	7881 ^{abc}	3051 ^{ab}	677 ^{a}
Biological Fertilizer OD	1607 ^{ab}	11827 ^{ab}	7298 ^{bcd}	2862 ^{bc}	**453 ^{e}**
Trichoderma OMG	1795 ^{a}	13304 ^{a}	**7045 ^{cd}**	**2861 ^{bc}**	499 ^{de}

* Different letters are describing significant differences (LSD; $p<0.05$)

5. 1. 4 Micronutrients content in aboveground biomass

Result of micronutrients (Fe, Cu, Zn, Mn, B, Ni) content in aboveground dry biomass are shown in (Table 5- 2). The Fe content was the highest in MUCI treatment (137 mg/kg) followed by RV 42 + MUCI (128 mg/kg), and Control (96.7 mg/kg) while the lowest content of Fe was recorded for Trichoderma OMG (59.4 mg/kg). Cu content was the highest in control (5.90 mg/kg) treatment followed by RhizoVital 45 (5.79 mg/kg) and RV42 (5.7 mg/kg), while the lowest was found in MUCI (4.8 mg/kg and RV42 + MUCI (4.2 mg/kg). RhizoVital 45 (31.9 mg/kg) treatment showed the highest Zn content in aboveground dry biomass whereas RV42 + MUCI (21.9 mg/kg) treatment showed the lowest content. For Mn content, the highest content was obtained by Proradix treatment (85.3 mg/kg). Then, the second and third highest content was found at MUCI (81.4 mg/kg) and RV 42 (79.9 mg/kg), respectively. However, the lowest Mn content was found at Trichoderma OMG (55.5 mg/kg).

B content was not significantly different within the treatments, but maximum content was recorded for RV42 + MUCI (19.3 mg/kg). Differences among Ni content were significant, but maximum content was found in treatment control (3.24 mg/kg) and MUCI (3.14 mg/kg). The minimum content of Ni was recorded for RV42 + MUCI (0.34 mg/kg) and SuperFifty (0.49 mg/kg).

Table 5- 2 Effect of biostimulants on micronutrients content in above ground dry biomass

Treatment	Fe(mg/kg)	Cu(mg/kg)	Zn(mg/kg)	Mn(mg/kg)	B(mg/kg)	Ni(mg/kg)
Control	96.7 ^{ab}	5.90 ^{a}	31.4 ^{a}	74.2 ^{a}	15.7 ^{ab}	3.24 ^{a}
Proradix	89.3 ^{ab}	5.48 ^{ab}	25.3 ^{bc}	85.3 ^{a}	13.5 ^{ab}	2.73 ^{ab}
RV42	68.5 ^{b}	5.73 ^{ab}	26.6 ^{abc}	79.9 ^{a}	14.7 ^{ab}	2.01 ^{cd}
RhizoVital 45	**62.7 ^{b}**	5.79 ^{ab}	31.9 ^{a}	78.9 ^{a}	16 ^{ab}	0.54 ^{e}
MUCI	137 ^{a}	**4.75 ^{bc}**	24.6 ^{bc}	81.4 ^{a}	**12.9 ^{b}**	3.14 ^{a}
RV42 + MUCI	128 ^{a}	**4.16 ^{c}**	**21.9 ^{c}**	74.5 ^{a}	19.3 ^{a}	**0.34 ^{e}**
SuperFifty	68.2 ^{b}	5.25 ^{ab}	26.8 ^{abc}	77.4 ^{a}	14.8 ^{ab}	0.49 ^{e}
NemaTec	67.1 ^{b}	5.15 ^{abc}	27.1 ^{abc}	77.5 ^{a}	14.3 ^{ab}	0.59 ^{e}
LamVita	73.5 ^{b}	5.71 ^{ab}	29.5 ^{ab}	76.3 ^{a}	14.6 ^{ab}	2.57 ^{b}
Biological Fertilizer OD	66.2 ^{b}	4.99 ^{abc}	24.0 ^{bc}	**58.9 ^{b}**	15.5 ^{ab}	2.50 ^{bc}
Trichoderma OMG	**59.4 ^{b}**	5.29 ^{ab}	27.4 ^{abc}	**55.5 ^{b}**	13.6 ^{ab}	1.85 ^{d}

* Different letters are describing significant differences (LSD; p<0.05)

5. 1. 5 Macronutrients uptake by aboveground biomass

We also measured macro and micronutrients uptake with aboveground biomass. For macronutrients, we calculated P, K, Ca, Mg, and S uptake to examine uptake influenced by biostimulants application. (Table 5- 3). According to our results, the highest P uptake with aboveground biomass was found at control (3.11mg) followed by Proradix (2.93 mg), and Trichoderma OMG (3.03 mg) respectively. The lowest P uptake was found at MUCI (2.31 mg) and RV42 + MUCI (2.05 mg). Application of Trichoderma OMG resulted in the highest K uptake among treatments (22.3 mg). Moreover, here also occurred significantly higher K uptake at control (21.5 mg) and Proradix (22 mg). The lowest K uptake was found at MUCI (14.6 mg/kg) and RV42 + MUCI (13.7 mg).

For Ca uptake, there were significant differences among treatments, but the highest Ca uptake was found in control (14.5 mg/kg). The second and third highest Ca uptake was found at RhizoVital 45 (13.3 mg) and NemaTec (13.1 mg), whereas the lowest uptake was found for MUCI (10.3 mg) and RV42 + MUCI (11.2 mg). The maximum uptake of Mg was also found at control (5.5 mg). After control, RhizoVital 45 (5.3 mg) and NemaTec (5.3 mg) showed higher Mg uptake than other treatments. The lowest uptake of Mg was found in MUCI (4.30 mg) and RV42 + MUCI (4.10 mg). For S uptake, NemaTec (1022 µg) and LamVita (1058 µg) was recorded the highest uptake compared to other treatments. Moreover, MUCI (673 µg) and RV42 + MUCI (788 µg) also resulted in the lowest in S uptake within treatments. According to our results, MUCI and RV42 + MUCI had negative effects at all macronutrients uptake by aboveground biomass.

Table 5- 3 Effect of biostimulants on macronutrients uptake by aboveground dry biomass

Treatment	P(mg)	K(mg)	Ca(mg)	Mg(mg)	S(µg)
Control	3.11 ^{a}	21.5 ^{ab}	14.5 ^{a}	5.54 ^{a}	1009 ^{ab}
Proradix	2.93 ^{ab}	22.0 ^{ab}	12.5 ^{bc}	4.89 ^{b}	936 ^{abc}
RV42	2.74 ^{abc}	19.7 ^{ab}	11.8 ^{bcd}	4.74 ^{bc}	898 ^{abc}
RhizoVital 45	2.63 ^{abc}	18.2 ^{abc}	13.3 ^{ab}	5.29 ^{ab}	923 ^{abc}
MUCI	**2.31 ^{cd}**	**14.6 ^{cd}**	**10.3 ^{d}**	**4.26 ^{cd}**	**673 ^{d}**
RV42 + MUCI	**2.05 ^{d}**	**13.7 ^{d}**	**11.2 ^{cd}**	**4.13 ^{d}**	**788 ^{cd}**
SuperFifty	2.53 ^{bcd}	17.7 ^{bcd}	12.6 ^{bc}	4.83 ^{bc}	1010 ^{ab}
NemaTec	2.57 ^{bc}	17.8 ^{bcd}	13.1 ^{ab}	5.26 ^{ab}	1022 ^{a}
LamVita	2.64 ^{abc}	17.9 ^{abcd}	12.3 ^{bc}	4.78 ^{bc}	1058 ^{a}
Biological Fertilizer OD	2.82 ^{ab}	20.9 ^{ab}	12.8 ^{abc}	4.99 ^{ab}	788 ^{cd}
Trichoderma OMG	3.03 ^{ab}	22.3 ^{a}	11.9 ^{bcd}	4.84 ^{bc}	835 ^{bcd}

* Different letters are describing significant differences (LSD; $p<0.05$)

5. 1. 6 Micronutrients uptake by aboveground biomass

Fe, Cu, Zn, Mn, B, and Ni contents were analyzed for further calculation of micronutrients uptake related to aboveground dry mass (Table 5- 4). The maximum uptake of Fe was recorded for MUCI (190 µg), followed by RV 42 + MUCI (177 µg), and Control (170 µg) while the lowest uptake was found at RhizoVital 45 (97.5 µg) and Trichoderma OMG

(99.7 µg). For Cu uptake, control (10.4 µg) resulted in the highest Cu uptake, followed by Proradix (10.1µg) and RV 42 (9.2 µg). The lowest Cu uptake was recorded at MUCI (6.5 µg) and RV42 + MUCI (5.9 µg). Zn uptake was highest in control (55 µg), followed by RhizoVital 45 (50 µg) and Proradix (47 µg) whereas the lowest uptake was found in MUCI (34 µg) and RV42 + MUCI (31.2 µg). Mn uptake was increased by Proradix application (159 µg). Control and RV 42 were also recorded as higher Mn uptake than other treatments. However, the lowest Mn uptake was found at Biological Fertilizer OD (105 µg) and Trichoderma OMG (93 µg). According to our results, there was no significant difference in B uptake by aboveground dry biomass. But the highest uptake was recorded for RV42 + MUCI (28.1 µg), control (28 µg), and Biological Fertilizer OD (28 µg). There was a significant difference in Ni uptake within treatments. Control (6 µg) resulted in the highest Ni uptake among treatments. Proradix (5.1 µg) and Biological Fertilizer OD (4.4 µg) also resulted the higher Ni uptake than other treatments. The lowest uptake was found in RV42 + MUCI (0.5 µg) and SuperFifty (0.7 µg). MUCI and RV42 + MUCI also showed negative effects on Cu and Zn uptake.

Table 5- 4 Effect of biostimulants on micronutrients uptake by aboveground dry biomass

Treatment	Fe(µg)	Cu(µg)	Zn(µg)	Mn(µg)	B(µg)	Ni(µg)
Control	**170** ^{ab}	10.4 ^{a}	54.9 ^{a}	132 ^{b}	27.6 ^{a}	5.70 ^{a}
Proradix	165 ^{b}	10.1 ^{ab}	47.0 ^{ab}	159 ^{a}	25.3 ^{a}	5.13 ^{ab}
RhizoVital 42 (RV42)	110 ^{b}	9.22 ^{abc}	43.1 ^{bc}	129 ^{bc}	23.8 ^{a}	3.26 ^{d}
RhizoVital 45	**97.5** ^{b}	9.00 ^{abc}	49.8 ^{ab}	123 ^{bc}	24.9 ^{a}	0.83 ^{e}
MUCI	190 ^{a}	**6.53** ^{de}	**33.8** ^{cd}	112 ^{bcd}	17.7 ^{a}	4.32 ^{bc}
RV42 + MUCI	177 ^{ab}	**5.88** ^{e}	**31.2** ^{d}	106 ^{bcd}	28.1 ^{a}	**0.47** ^{e}
SuperFifty	105 ^{b}	8.05 ^{cd}	41 ^{bcd}	119 ^{bc}	22.8 ^{a}	**0.72** ^{e}
NemaTec	101 b	8.54 ^{bc}	45.4 ^{ab}	129 ^{bc}	24.0 ^{a}	0.99 ^{e}
LamVita	114 ^{b}	8.92 ^{abc}	46.3 ^{ab}	120 ^{bc}	22.9 ^{a}	4.01 ^{cd}
Biological Fertilizer OD	117 ^{b}	8.79 ^{abc}	42.1 ^{bc}	**105** ^{cd}	27.6 ^{a}	4.39 ^{bc}
Trichoderma OMG	**99.7** ^{b}	8.92 ^{abc}	46.5 ^{ab}	**92.6** ^{d}	22.9 ^{a}	3.12 ^{d}

* Different letters are describing significant differences (LSD; $p<0.05$)

5. 2 Combined application of rock phosphate with different biostimulants treatments

5. 2. 1 Plant height

Plant height was measured at two weeks and five weeks after sowing during our experiment (Figure 5- 4 and Figure 5- 5). At two weeks after sowing, there was a significant difference among treatments. The highest growth was recorded with RV42 + RP (25.2 cm), followed by RhizoVital 45 + RP (24.3 cm) and NemaTec (24.1 cm). The lowest growth in plant height was recorded for MUCI + RP (21 cm) and RV42 + MUCI + RP (20 cm). However, there was no significant difference among treatments measured five weeks after sowing. The highest plant height was found in NemaTec (74 cm). When compared to other treatments, RV42 (73 cm) and RhizoVital 45 (71.1 cm) were also like NemaTec (73 cm).

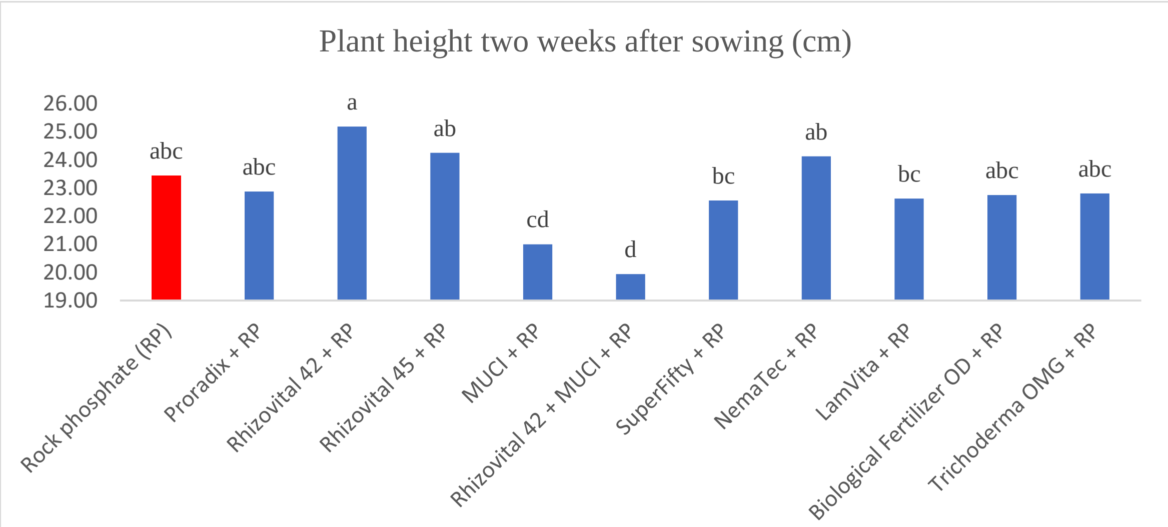

Figure 5- 4 Plant height affected by RP + different bio-stimulants

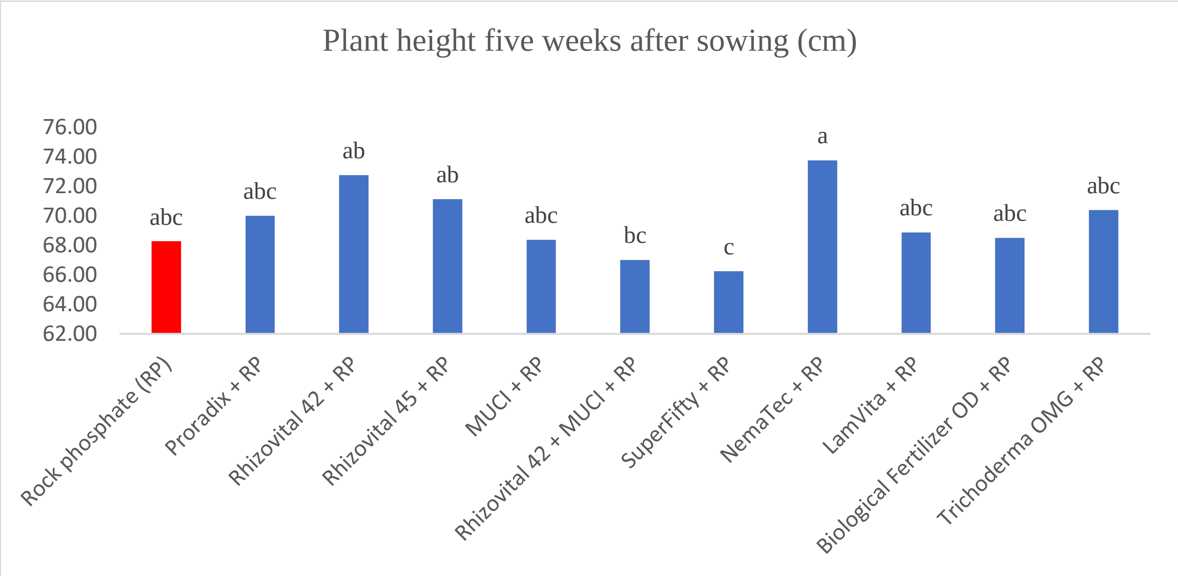

Figure 5- 5 Plant height affected by RP + different biostimulants

5. 2. 2 Aboveground biomass weight

After harvest, the aboveground biomass of plants was air-dried and weight for analysis (Figure 5- 6). Different treatments significantly ($p < 0.05$) influenced aboveground biomass dry weight. Proradix increased significantly aboveground biomass within treatments (1.9 g). After Proradix, the maximum aboveground biomass dry weight was found at control (1.8 g) and Biological Fertilizer OD (1.8 g). The lowest aboveground biomass dry weight was found at MUCI (1.4 g) and RV42 + MUCI (1.4 g). Therefore, the combined application of MUCI with phosphate rock and RV42 + MUCI with phosphate rock had also negative effects on aboveground biomass weight.

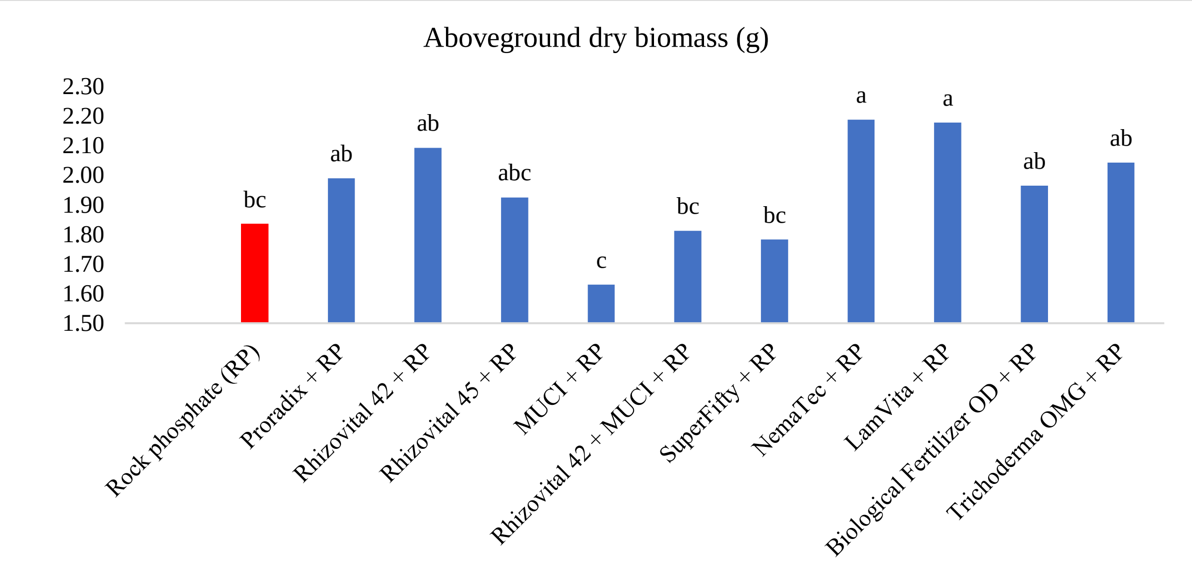

Figure 5- 6 Above biomass affected by RP + different bio stimulants

5. 2. 3 Macronutrients content in above ground biomass

According to our result, P content in aboveground biomass was highest in RhizoVital 45 (1648 mg/kg) followed by Biological Fertilizer OD (1642 mg/kg) and RV42 (1594 mg/kg) (Table 5- 5). The lowest P content was found in RP + Trichoderma OMG (1335 mg/kg) and RV42 + MUCI (1248 mg/kg). The highest K content was recorded for MUCI (13142 mg/kg) followed by RV42 + MUCI (13055 mg/kg) and SuperFifty (11798 mg/kg). The lowest K content was found in Proradix (9048 mg/kg) and RV42 (9318 mg/kg). RhizoVital 45 (6611 mg/kg), RV42 (6554 mg/kg) and Biological Fertilizer OD (6522 mg/kg) resulted in highest Ca content in aboveground biomass while the lowest content was found at Trichoderma OMG (4448 mg/kg) and RV42 + MUCI (4246 mg/kg).

The differences between Mg and S content were not significant. However, the highest Mg content was recorded for Biological Fertilizer OD (2947 mg/kg), RV42 (2929 mg/kg) and RhizoVital 45 (2845 mg/kg), respectively. The lowest Mg content was found at RV42 + MUCI (1968 mg/kg) and Trichoderma OMG (2254 mg/kg). For S content, the highest content was recorded for SuperFifty (587 mg/kg) and RhizoVital 45 (583 mg/kg) whereas the lowest was found in RV42 + MUCI (435 mg/kg) and MUCI (462 mg/kg). Generally, although RV42 + MUCI and MUCI had negative effects on almost every parameter, K content in aboveground biomass was highest within this treatment.

Table 5- 5 Effect of combined application of phosphate rock with different biostimulants on macronutrients content in aboveground biomass

Treatment	P(mg/kg)	K(mg/kg)	Ca(mg/kg)	Mg(mg/kg)	S(mg/kg)
Control (RP only)	1563 [a]	11326 [ab]	6074 [a]	2663 [ab]	568 [ab]
Proradix +RP	1549 [a]	**9048 [b]**	6403 [a]	2825 [a]	501[abc]
RV42 +RP	1594 [a]	**9318 [b]**	6554 [a]	2929 [a]	492[abc]
RhizoVitall 45 +RP	1648 [a]	11374 [ab]	6611 [a]	2845 [a]	583 [a]
MUCI +RP	1494 [a]	13142 [a]	5389 [abc]	2496 [ab]	462 [abc]
RV42 + MUCI +RP	**1248 [a]**	13055 [a]	**4246 [c]**	**1968 [b]**	**435 [bc]**
SuperFifty+RP	1561 [a]	11798 [ab]	5887 [abc]	2628 [ab]	587 [a]
NemaTec +RP	1587 [a]	9590 [b]	5927 [ab]	2858 [ab]	519 [abc]
LamVita +RP	1533 [a]	9896 [b]	5713 [abc]	2589 [ab]	573 [ab]
Biological Fertilizer OD +RP	1642 [a]	9835 [b]	6522 [a]	2947 [a]	569 [ab]
Trichoderma OMG +RP	**1335 [a]**	11133 [ab]	**4448 [bc]**	**2254 [ab]**	**416 [c]**

* Different letters are describing significant differences (LSD; $p<0.05$)

5. 2. 4 Micronutrients content in aboveground biomass

As shown in Table 5- 6, Fe content was significantly different within treatments. The highest Fe contents were found in Control (113 mg/kg) and Proradix (104 mg/kg). However, the lowest Fe contents were recorded for RV42 + MUCI (47 mg/kg) and MUCI (47 mg/kg). The Cu content was not significantly different within the treatments, but the highest content was found in Biological Fertilizer OD (5.3 mg/kg), followed by NemaTec + RP (5.2 mg/kg) and Control (5.1 mg/kg). The lowest Cu contents were recorded at MUCI (4.1 mg/kg), Trichoderma OMG (4 mg/kg) and RV42 + MUCI (3.5 mg/kg). Zn contents were highest in Biological Fertilizer OD (27 mg/kg), RhizoVitall 45 (27 mg/kg) and RV 42 (26.3 mg/kg), while lowest was found at RV42 + MUCI (15.1 mg/kg) and MUCI (20 mg/kg). For Mn, the highest content was found in Biological Fertilizer OD (75 mg/kg), NemaTec (68 mg/kg) and LamVita (68 mg/kg). Within treatments, the lowest Mn contents were recorded at

Trichoderma OMG (48 mg/kg) and RV42 + MUCI (49 mg/kg). The highest B content was occurred at LamVita (17 mg/kg), while the lowest was recorded for Trichoderma OMG (7.4 mg/kg). Ni content did not show significant differences within the treatments, but the highest was found at Control (3.4 mg/kg) followed by Biological Fertilizer OD (2.2 mg/kg) and Proradix (2.1 mg/kg) and the lowest was found at RV42 + MUCI (0.1 mg/kg) and LamVita (0.5 mg/kg).

Table 5- 6 Effect of combined application of phosphate rock with different biostimulants on micronutrients content in above ground biomass

Treatment	Fe(mg/kg)	Cu(mg/kg)	Zn(mg/kg)	Mn(mg/kg)	B(mg/kg)	Ni(mg/kg)
Control (RP only)	113 [a]	5.07 [ab]	21.4 [ab]	62.1 [abcd]	13.7 [ab]	3.39 [a]
Proradix +RP	104 [a]	4.48 [abc]	26.1 [a]	60.2 [abcd]	15.4 [ab]	2.14 [b]
RV42 +RP	70.3 [b]	4.80 [abc]	26.3 [a]	62.1 [abcd]	15.1 [ab]	1.79 [bc]
RhizoVitall 45 +RP	72 [b]	4.85 [abc]	26.9 [a]	64.0 [abc]	15.5 [ab]	1.64 [bc]
MUCI +RP	**46.7 [b]**	4.08 [abc]	**19.8 [ab]**	56.6 [bcd]	13.6 [ab]	1.50 [bc]
RV42 + MUCI +RP	**46.8 [b]**	**3.53 [bc]**	**15.1 [b]**	**48.7 [cd]**	**9.6 [cd]**	**0.11 [d]**
SuperFifty +RP	53.5 [b]	4.90 [ab]	22.9 [a]	62.8 [abcde]	15 [ab]	1.90 [bc]
NemaTec +RP	51.0 [b]	5.15 [ab]	23.4 [a]	68.2 [ab]	12.6 [bc]	2.08 [b]
LamVita +RP	57.2 [b]	4.84 [abc]	24.7 [a]	67.7 [ab]	16.7 [a]	**0.47 [d]**
Biological Fertilizer OD +RP	55.4 [b]	5.32 [a]	27 [a]	74.7 [a]	14.0 [ab]	2.18 [b]
Trichoderma OMG +RP	51.4 [b]	**3.90 [c]**	20.9 [ab]	**47.6 [d]**	**7.4 [d]**	1.31 [c]

* Different letters are describing significant differences (LSD; $p<0.05$)

5. 2. 5 Macronutrients uptake by aboveground biomass

P uptake in aboveground biomass was highest in NemaTec (3.5 mg), followed by RV42 (3.3 mg) and LamVita (3.3 mg), while the lowest was found at MUCI (2.5 mg) and RV42 + MUCI (2.2 mg) (Table 5- 7). The highest K uptake was found at RV42 + MUCI (24 mg), Trichoderma OMG (23 mg) and LamVita (22 mg), whereas the lowest K uptake was recorded at Proradix (18 mg), and Biological Fertilizer OD (19 mg). For Ca uptake, the highest were found at RV 42 (14 mg), NemaTec (13 mg) and Biological Fertilizer OD (13 mg). However, the lowest Ca uptakes were recorded for MUCI (9 mg), Trichoderma OMG (9 mg) and RV42 + MUCI (8 mg). Mg uptake was increased by NemaTec (6.2 mg), RV42 (6.1 mg) and Biological Fertilizer OD (5.7 mg). However, the lowest uptake was found at MUCI (4.2 mg) and RV42 + MUCI (3.5 mg). The highest S uptake was found at LamVita (1232 µg), followed by NemaTec (1131 µg) and RhizoVital 45 (1116 µg). The lowest S uptake was recorded for MUCI (768 µg) and RV42 + MUCI (780 µg).

Table 5- 7 Effect of combined application of phosphate rock with different biostimulants on macronutrients uptake by aboveground biomass

Treatment	P(mg)	K(mg)	Ca(mg)	Mg(mg)	S(µg)
Control (RP only)	2.87 ^{abcd}	20.9 ^{ab}	11.1 ^{abc}	4.86 ^{bcde}	1036 ^{ab}
Proradix +RP	3.08 ^{abc}	**18.0 b**	12.7 ^{ab}	5.61 ^{abcd}	996 ^{abc}
RV42 +RP	3.33 ^{ab}	19.5 ^{ab}	13.7 ^{ab}	6.10 ^{ab}	1026 ^{abc}
RhizoVitall 45 +RP	3.15 ^{abc}	21.9 ^{ab}	12.6 ^{ab}	5.43 ^{abcde}	1116 ^{a}
MUCI +RP	**2.49 ^{cd}**	20.8 ^{ab}	9.00 ^{cd}	**4.17 ^{ef}**	**768 ^{c}**
RV42 + MUCI +RP	**2.24 ^{d}**	23.5 ^{a}	**7.65 ^{d}**	**3.54 ^{f}**	**780 ^{c}**
SuperFifty +RP	2.78 ^{abc}	21 ^{ab}	10.5 ^{bc}	4.68 ^{cdef}	1045 ^{ab}
NemaTec +RP	3.47 ^{a}	21.0 ^{ab}	12.9 ^{ab}	6.22 ^{a}	1131 ^{a}
LamVita +RP	3.28 ^{abc}	21.5 ^{ab}	12.3 ^{ab}	5.57 ^{abcd}	1232 ^{a}
Biological Fertilizer OD +RP	3.16 ^{abc}	**19.2 ^{ab}**	12.7 ^{ab}	5.73 ^{abc}	1097 ^{a}
Trichoderma OMG +RP	2.62 ^{bcd}	22.6 ^{ab}	**8.78 ^{cd}**	4.44 ^{def}	815 ^{bc}

* Different letters are describing significant differences (LSD; $p<0.05$)

5. 2. 6 Micronutrients uptake by aboveground biomass

Fe, Cu, Zn, Mn, B, and Ni were analyzed also to examine micronutrient uptake under different biostimulants applications (Table 5- 8).

Table 5- 8 Effect of combined application of phosphate rock with different biostimulants on micronutrients uptake by above ground biomass

Treatment	Fe(µg)	Cu(µg)	Zn(µg)	Mn(µg)	B(µg)	Ni(µg)
Control (RP only)	207 ^{a}	9.33 ^{abc}	38.9 ^{bc}	114 ^{abcd}	25.1 ^{bc}	6.29 ^{a}
Proradix + RP	204 ^{ab}	8.92 ^{abcd}	51.5 ^{ab}	119 ^{abcd}	30.5 ^{ab}	4.22 ^{b}
RV42 +RP	148 ^{abc}	10.03 ^{abc}	54.9 ^{a}	130 ^{abc}	31.3 ^{ab}	3.76 ^{bc}
RhizoVital 45 +RP	143 ^{bcd}	9.28 ^{abc}	51.1 ^{ab}	123 ^{abcd}	29.8 ^{abc}	3.12 ^{bc}
MUCI +RP	**78.1 ^{e}**	**6.78 ^{de}**	**33.1 ^{c}**	**94.9 ^{cd}**	22.7 ^{cd}	2.55 ^{bc}
RV42 + MUCI + RP	**83.7 ^{cde}**	**6.35 ^{e}**	**27.1 ^{c}**	**88 ^{d}**	**17.4 ^{de}**	**0.19 ^{d}**
SuperFifty +RP	95.0 ^{cde}	8.73 ^{bcde}	41.1 ^{abc}	112 ^{bcd}	26.6 ^{bc}	3.39 ^{bc}
NemaTec +RP	111 ^{cde}	11.2 ^{a}	50.9 ^{ab}	148 ^{a}	27.7 ^{bc}	4.58 ^{b}
LamVita + RP	122 ^{cde}	10.4 ^{ab}	53.2 ^{a}	148 ^{a}	35.7 ^{a}	**0.92 ^{d}**
Biological Fertilizer OD +RP	106 ^{cde}	10.3 ^{ab}	52.6 ^{ab}	146 ^{ab}	27.0 ^{bc}	4.28 ^{b}
Trichoderma OMG + RP	99.8 ^{cde}	7.62 ^{cde}	40.90 ^{abc}	**94.79 ^{cd}**	**14.7 ^{e}**	2.53 ^{c}

* Different letters are describing significant differences (LSD; $p<0.05$)

According to results, big differences were found in micronutrients uptake by aboveground biomass under combined application of rock phosphate with different biostimulants. Fe uptake by aboveground biomass was highest for Control (207 µg), followed by Proradix (204 µg) and RV 42 (148 µg), while the lowest was found at MUCI (78 µg) and RV42 + MUCI (84 µg). Cu uptake was the highest at NemaTec (11.2 µg). The second and third highest uptake was obtained for LamVita (10.4 µg) and Biological Fertilizer OD (10.4 µg), whereas the lowest was found at MUCI (7 µg) and RV42 + MUCI (6.4 µg). Although the highest Zn uptake was found at RV 42 (55 µg), LamVita (53 µg) and Biological Fertilizer OD (53 µg), the lowest was obtained for MUCI (33.1 µg) and RV42 + MUCI (27.1 µg). For Mn uptake, the highest was found at NemaTec (148 µg), followed by LamVita (148 µg) and Biological Fertilizer OD (146 µg), whereas the lowest was recorded with MUCI (95 µg), RV42 + MUCI (88 µg) and Trichoderma OMG (95 µg). Boron uptake was the highest in LamVita (36 µg), followed by RV42 (31.3 µg) and Proradix (31 µg). The lowest uptake was found at MUCI (23 µg), RV42 + MUCI (17.4 µg), and Trichoderma OMG (15 µg). Control (6.3 µg) resulted in the highest Ni uptake among treatments, followed by NemaTec (4.6 µg) and Biological Fertilizer OD (4.3 µg), while the lowest was recorded with RV42 + MUCI (0.2 µg) and LamVita (0.92 µg).

5. 3 Comparison of non-fertilized with RP treatments

5.3.1 Plant height (after two weeks)

When compared biostimulants themselves or with rock phosphate treatments (Figure 5- 7), the highest plant height was found in rock phosphate + biostimulants application at two weeks after sowing. The highest plant height was recorded for RV 42 + RP (25.2 cm), followed by RV 45 + RP (24.3 cm) and NemaTec (24.1 cm).

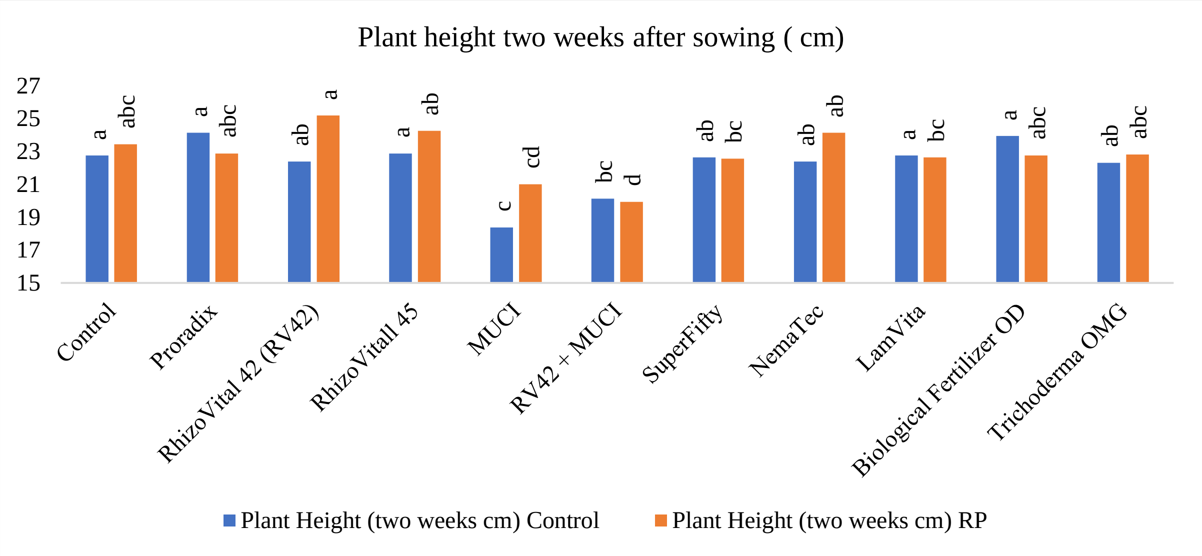

Figure 5- 7 Comparison of plant height between non fertilized and RP treatments

5.3.2 Plant height (after five weeks)

At five weeks after sowing, RP + biostimulants application showed significantly higher plant height compared to the only application of biostimulants. In this experiment, the highest plant height was found at NemaTec + RP (74 cm) and RV 42 + RP (73 cm).

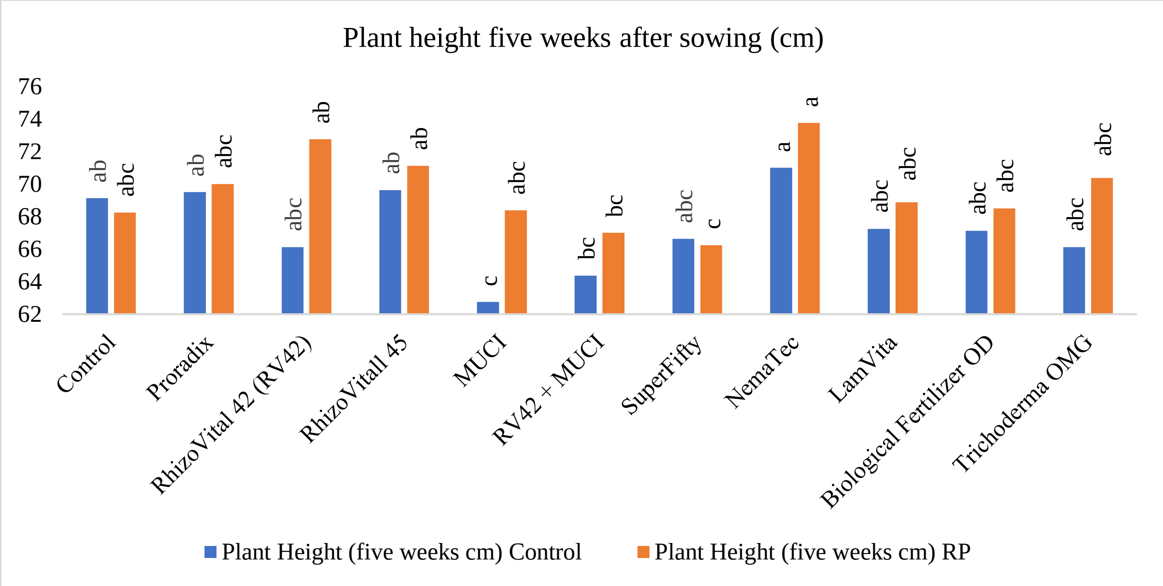

Figure 5- 8 Comparison of plant height between non fertilized and RP treatments

5.3.3 Aboveground biomass weight

The application of RP + biostimulants resulted in higher plant weight than the only application of biostimulants. The highest results were recorded for NemaTec + RP (2.2 g), LamVita (2.2 g) and RV 42 + RP (2.1 cm).

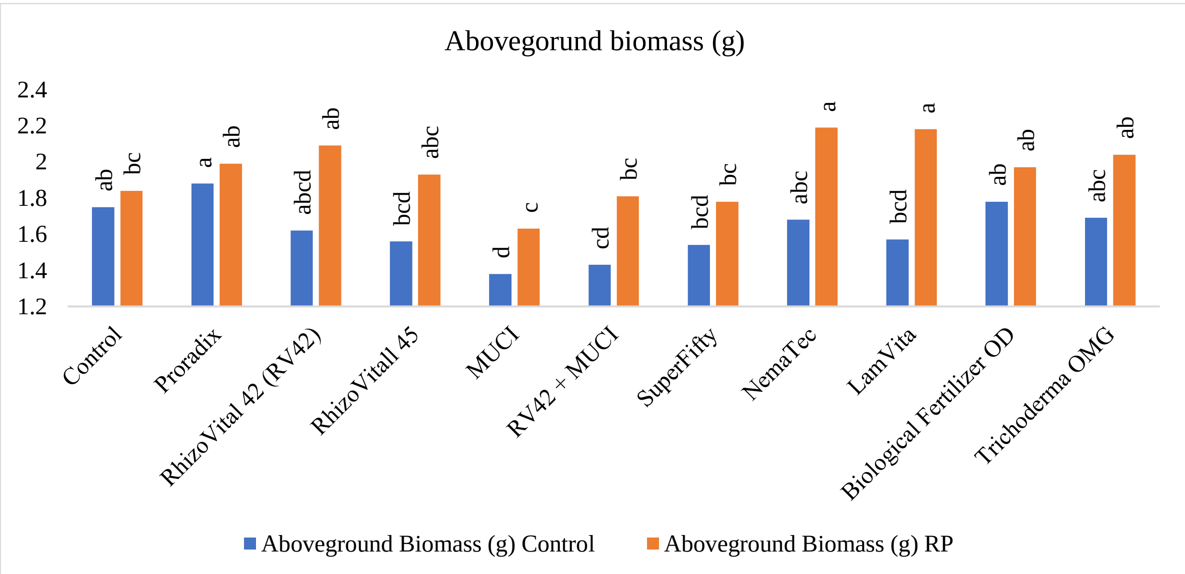

Figure 5-9 Comparison of aboveground weight between non fertilized and RP treatments

5.3.4 Comparison of macronutrients content between non fertilized and RP treatments

When compared biostimulants only with RP + biostimulants application, the highest P content was found in only biostimulants application (Table 5- 9). The highest P values were recorded for Trichoderma OMG followed by Control (soil only) and RV 42 between non fertilized and RP treatments. The lowest P values was recorded for RV42 + MUCI + RP, Trichoderma OMG + RP, and LamVita + RP. For K content, the highest values were found for Trichoderma OMG, MUCI + RP, and RV42 + MUCI +RP between two applications. The lowest K was recorded for RV 42 + RP, Biological Fertilizer OD + RP and Proradix + RP. Ca content was also higher in biostimulant application than RP + biostimulants application. The highest Ca content was recorded for RhizoVital 45, control (soil only) and SuperFifty between two application while the lowest Ca content was recorded for at RV42 + MUCI + RP, Trichoderma OMG + RP and MUCI + RP. The biostimulants application resulted higher Mg content than RP + biostimulants. The maximum Mg content was found at RhizoVital 45, control (soil only) and SuperFifty between two applications. The lowest Mg content was found at RV42 + MUCI + RP, Trichoderma OMG + RP and MUCI + RP. S content was also highest in biostimulants applications. LamVita, NemaTec and SuperFifty were highest in S content. The lowest S content was found at Trichoderma OMG + RP, RV42 + MUCI + RP, and Biological Fertilizer OD. According to results, only biostimulants application was higher in macronutrients content than the combined application of RP with biostimulant applications.

5.3.5 Comparison of micronutrients content in biomass between non fertilized and RP treatments

Table 5- 10 shows a comparison of micronutrients content between two applications**.** The highest Fe content was found at the biostimulants only application. MUCI, RV42 + MUCI and Control (RP only) were highest in Fe content between two applications while the lowest content was found at MUCI + RP, RV42 + MUCI + RP and NemaTec + RP. Cu content was higher in biostimulants application than RP + biostimulants. Control (soil only), RhizoVital 45, and RV 42 were recorded as highest Cu content between two applications. The lowest Cu content was found at RV42 + MUCI + RP, Trichoderma OMG + RP, and MUCI + RP. RhizoVital 45, Control (soil only) and LamVita showed the highest Zn content between two applications while the lowest content found at RV42 + MUCI + RP, MUCI + RP and Trichoderma OMG + RP. Proradix, MUCI and RV 42 were also highest in Mn content between two applications while the lowest content was found at Trichoderma OMG +

RP, RV42 + MUCI + RP, and MUCI + RP. The highest B content was found at RV42 + MUCI, RhizoVital 45 and LamVita + RP between two applications while the lowest content was found at Trichoderma OMG + RP, RV42 + MUCI + RP, and MUCI. Control (Soil + RP only) was highest in Ni content followed by Control (soil only) and MUCI between two applications. The lowest Ni content was recorded for LamVita + RP and RV 42 + MUCI. From the point of nutrients content view, the application of biostimulants was more effective than the combined application of RP + biostimulants. It is probably due to the dilution effect due to the higher biomass yields at the RP fertilized treatments.

5.3.6 Comparison of macronutrients uptake by aboveground biomass between non fertilized and RP treatments

The application of biostimulants and combined application of RP + biostimulants were compared to investigate the most effective treatments. The P uptake was higher in RP + bio stimulants than biostimulants application. The highest P uptake was found at RP + NemaTec, followed by RV 42 + RP and LamVita + RP while the lowest uptake was found at RV42 + MUCI, RV42 + MUCI + RP and MUCI. For K uptake, the highest was found at RV42 + MUCI + RP, Trichoderma OMG + RP and Trichoderma OMG between application while the lowest uptake was found at RV42 + MUCI, MUCI, and SuperFifty. The highest Ca uptake was found at Control (soil only), RV42 + RP and RhizoVital 45 between two applications while the lowest uptake was found at RV42 + MUCI + RP, Trichoderma OMG + RP and MUCI + RP. Mg uptake was highest in RP + biostimulants application. RP + NemaTec, RP + RV42 and RP + Biological Fertilizer OD showed highest Mg uptake between two applications while the lowest was found at RV42 + MUCI + RP, RV 42 + MUCI and MUCI + RP. S uptake was also higher in RP + biostimulants application than biostimulants application. The highest S uptake was found at LamVita + RP, NemaTec + RP and RhizoVital 45 + RP while the lowest was found at MUCI, MUCI + RP and RV42 + MUCI + RP. According to results, the combined application of RP with biostimulants resulted in higher macronutrients uptake by aboveground biomass than biostimulants application.

5.3.7 Comparison of micronutrients uptake by aboveground biomass non fertilized and RP treatments

When compared to the biostimulants only or with RP + biostimulants application to examine the most effective treatments, Fe uptake by aboveground biomass was highest in Control (RP only) followed Proradix + RP and MUCI, while the lowest was found at MUCI + RP, RV42 + MUCI + RP, and NemaTec. For Cu uptake, NemaTec + RP, LamVita + RP and Control (soil only) resulted in highest Cu uptake between two applications while the lowest was found at RV42 + MUCI + RP, Trichoderma OMG + RP and MUCI + RP. The highest Zn uptake was found at RV42 + RP, followed by Control (soil only) and LamVita + RP. The lowest Zn uptake was found at RV42 + MUCI + RP, MUCI + RP and Trichoderma OMG + RP. Mn uptake was highest in Proradix followed by NemaTec + RP and LamVita + RP while the lowest was found at Trichoderma OMG + RP, RV42 + MUCI + RP and MUCI + RP. For B uptake, the highest uptake was found for RP + LamVita, RP + RV42 and RP + Proradix while the lowest was found at Trichoderma OMG + RP, RV42 + MUCI + RP and MUCI. For Ni uptake, the highest uptake was found at Control (RP only) followed by Control (soil only) and Proradix while the lowest was found at RV42 + MUCI + RP, RV42 + MUCI + RP and Lamvita. Micronutrients uptake was also higher in the combined application of RP + biostimulants than only biostimulants application.

Table 5 -9 Comparison of macronutrients content among biostimulants and biostimulants + RP treatments

Treatment	P(mg/kg)		K(mg/kg)		Ca(mg/kg)		Mg(mg/kg)		S(mg/kg)	
	Control	RP	Control	RP	Control	RP	Control	RP	Control	RP
Control	1767 a **	1563 a	12173 ab**	11326 ab	8279 ab **	6074 a	3175 ab **	2663 ab	570 abcde	568 ab
Proradix	1579 ab	1549 a	11910 ab	**9048 b**	6704 d	6403 a	2625 c	2825 a	504 cde	501 abc
RV42	1701 ab *	1594 a *	12108 ab *	**9318 b**	7349 bcd	6554 a **	2953 bc	2929 a **	557 bcde	492 abc
RhizoVital 45	1689 ab	1648 a ***	11730 ab	11374 ab	8536 a ***	6611 a ***	3395 a ***	2845 a *	593 abcd	583 a**
MUCI	1682 ab	1494 a	10630 b	13142 a ***	7496 bcd	**5389 abc**	3103 ab	**2495 ab**	490 de	461 abc
RV42 + MUCI	1447 b	**1248 a**	9703 b	13055 a **	7896 abc	**4246 c**	2903 bc	**1968 b**	559 bcde	**435 bc**
SuperFifty	1651 ab	1561 a	11603 ab	11798 ab *	8205 abc *	5887 abc	3155 ab *	2628 ab	659 ab **	587 a***
NemaTec	1536 ab	1587 a	10650 b	9590 b	7793 abc	5927 ab	3138 ab	2858 ab	614 abc *	519 abc
LamVita	1688 ab	**1533 a**	11490 ab	9896 b	7881 abc	5713 abc	3051 ab	2589 ab	677 a ***	573 ab *
Biological Fertilizer OD	1607 ab	1642 a **	11827 ab	**9835 b**	7298 bcd	6522 a *	2862 bc	2947 a ***	**453 e**	569 ab
Trichoderma OMG	1795 a ***	**1335 a**	13304 a ***	11133 ab	7046 cd	**4448 bc**	2861 bc	**2254 ab**	499 cde	**416 c**

(***) = highest value followed by 2nd (**) and 3rd (*) LSD ($p<0.05$)

Table 5- 10 Comparison of micronutrient content among biostimulants and biostimulants + RP treatments

Treatment	Fe (mg/kg)		Cu (mg/kg)		Zn (mg/kg)		Mn (mg/kg)		B (mg/kg)		Ni (mg/kg)	
	Control	RP	Control	RP	Control	RP	Control	RP	Control	RP	Control	RP
Control	**96.7 a ***	113 a ***	5.90 a ***	5.07 ab *	31.4 a **	21.4 ab	74.2 a	62.1 abcd	15.7 ab *	13.7 ab	3.24 a ***	3.39 a ***
Proradix	89.3 b	104 a **	5.48 ab	4.48 abc	25.3 bc	26.1 a	85.3 a ***	60.2 abcd	13.5 ab	15.4 ab *	2.73 ab *	2.14 b *
RV42	68.5 b	70.3 b	5.73 ab *	4.80 abc	26.6 abc	26.3 a	79.9 a *	abcd	14.7 ab	15.1 ab	2.01 cd	1.79 bc
RhizoVital 45	62.7 b	72 b *	5.79 ab **	4.85 abc	31.9 a ***	26.9 a	78.9 a	64.0 abc	16 ab **	15.5 ab **	0.54 e	1.64 bc
MUCI	137 b ***	**46.7 b**	4.75 bc	**4.08 abc**	24.6 bc	**19.8 ab**	81.4 a **	**56.60 bcd**	**12.9 b**	13.6 ab	3.14 a **	1.50 bc
RV42 + MUCI	128 b **	**46.8 b**	4.16 c	**3.53 c**	22 c	**15.1 b**	74.5 a	**48.7 cd**	19.3 a ***	**9.62 cd**	**0.34 e**	**0.11 d**
SuperFifty	68.2 b	53.5 b	5.25 ab	4.90 ab	26.8 abc	22.9 a **	77.4 a	62.8 abcde	14.84 ab	15 ab	0.49 e	1.90 bc
NemaTec	67.1 b	**51.0 b**	5.15 abc	5.15 ab **	27.1 abc	23.4 a	77.5 a	68.2 ab **	14.3 ab	12.6 bc	0.59 e	2.08 b
LamVita	73.5 b	57.2 b	5.71 ab	4.84 abc	29.5 ab *	24.7 a *	76.3 a	67.7 ab *	14.6 ab	16.7 a ***	2.57 b	**0.47 d**
Biological Fertilizer OD	66.2 b	55.4 b	4.99 abc	5.32 a ***	24.0 bc	27 a ***	58.9 b	74.6 a ***	15.5 ab	14.0 ab	2.50 bc	2.18 b **
Trichoderma OMG	59.4 b	51.4 b	5.29 ab	**3.90 bc**	27.4 abc	**20.9 ab**	55.5 b	**47.6 d**	13.6 ab	**7.37 d**	1.85 d	1.31 c

(***) = highest value followed by 2nd (**) and 3rd (*) LSD (p<0.05)

Table 5- 11 Comparison of macronutrients uptake among biostimulants and biostimulants + RP treatments

Treatment	P(mg)		K(mg)		Ca(mg)		Mg(mg)		S(µg)	
	Control	RP	Control	RP	Control	RP	Control	RP	Control	RP
Control	3.11 a ***	2.87 abcd	21.5 ab *	20.9 ab	14.5 a ***	11.1	5.54 a ***	4.86 bcde	1009 ab	1036 ab
Proradix	2.93 ab *	3.08 abc	22 ab **	18.03 b	12.5 bc	12.7	4.89 b	5.61	936 abc	996 abc
RV42	2.74 abc	3.33 ab **	19.7 ab	19.5 ab	11.8 bcd	13.7 ab ***	4.74 bc	6.10 ab **	898 abc	1026 abc
RhizoVitall 45	2.63 abc	3.15 abc	18.2 abc	21.9 ab	13.3 ab **	12.6 ab	5.29 ab **	5.4 abcde	923 abc	1116 a *
MUCI	**2.31 cd**	2.49 cd	**14.6 cd**	20.8 ab	10.3 d	**9.00 cd**	4.26 cd	**4.17 ef**	**673 d**	**768 c**
RV42 + MUCI	**2.05 d**	**2.24 d**	**13.7 d**	23.5 a ***	11.2 cd	**7.65 d**	**4.13 d**	**3.54 f**	788 cd	**780 c**
SuperFifty	2.53 bcd	2.78 abc	**17.7 bcd**	21 ab	12.6 bc	10.51 bc	4.83 bc	4.68 cdef	1010 ab *	1045 ab
NemaTec	2.57 bc	3.47 a ***	17.8 bcd	21 ab	13.1 ab *	12.9 ab **	5.26 ab *	6.22 a ***	1022 a **	1131 a **
LamVita	2.64 abc	3.28 abc *	17.9 abcd	21.5 ab *	12.3 bc	12.3 ab	4.78 bc	5.57 abcd	1058 a ***	1232 a ***
Biological Fertilizer OD	2.82 ab	3.16 abc	20.9 ab	19.2 ab	12.8 abc	12.7 ab *	4.99 ab	5.73 abc *	788 cd	1097 a
Trichoderma OMG	3.03 ab **	2.62 bcd	22.3 a ***	22.6 ab **	11.9 bcd	**8.78 cd**	4.84 bc	4.44 def	835 bcd	815 bc

(***) = highest value followed by 2nd (**) and 3rd (*) LSD (p<0.05)

Table 5- 12 Comparison of micronutrients uptake among biostimulants and biostimulants + RP treatments

Treatment	Fe (µg)		Cu (µg)		Zn (µg)		Mn (µg)		B (µg)		Ni (µg)	
	Control	Rp	Control	Rp	Control	Rp	Control	Rp	Control	Rp	Control	Rp
Control	170 ab *	207 a ***	10.4 a ***	9.33 abc	54.9 ***	39 bc	132 b **	114 abcd	27.6 a **	25.1 bc	5.70 a ***	6.29 a ***
Proradix	165 b	204 ab **	10.1 ab **	8.92 abcd	47 ab *	51.5 ab	159 a ***	119 abcd	25.3 a	30.5 ab *	5.13 ab **	4.22 b
RV42	110 b	148 abc *	9.22 abc *	10.0 abc	43.1 bc	54.9 a ***	129 bc *	130 abc	23.8 a	31.3 ab **	3.26 d	3.76 bc
RhizoVital 45	98 b	144 bcd	9.00 abc	9.28 abc	49.8 ab **	51.1 ab	123 bc	123 abcd	24.9 a	29.8 abc	0.83 e	3.12 bc
MUCI	190 a ***	78.1 e	6.53 de	6.78 de	33.8 cd	33.1 c	112 bcd	95 cd	17.7 a	22.7 cd	4.32 bc	2.55 bc
RV42 + MUCI	177 ab **	83.7 cde	5.88 e	6.35 e	31.2 d	27.1 c	106 bcd	87.9 d	28.1 a ***	17.4 de	0.47 e	0.19 d
SuperFifty	104.66 b	95 cde	8.05 cd	8.73 bcde	50 bcd	41.1 abc	119 bc	112. bcd	22.8 a	26.6 bc	0.72 e	3.39 bc
NemaTec	101 b	111 cde	8.54 bc	11.2 a ***	45.4 ab	50.9 ab	129 bc	148 a ***	24 a	27.7 bc	0.99 e	4.58 b **
LamVita	114 b	123cde	8.92 abc	10.4 ab **	46.3 ab	53.2 a **	120 bc	148 a **	22.9 a	35.7 a ***	4.01 cd	0.92 d
Biological Fertilizer OD	117 b	106cde	8.79 abc	10.3 ab *	42.1 bc	52.6 ab *	105 cd	146 ab *	27.6 a *	27 bc	4.39 bc *	4.28 b *
Trichoderma OMG	100 b	99.9 cde	8.92 abc	7.62 cde	46.5 ab	40.9 abc	92.6 d	94.8 cd	22.9 a	14.7 e	3.12 d	2.53 c

(***) = highest value followed by 2nd (**) and 3rd (*) LSD (p<0.05)

5. 4 General evaluation of all treatments

There were three types of biostimulnats used in our experiment, bacterial, fungal, and algae extract groups. According to our result data, control treatment (not applied biostimulants) was the most effective treatment in our experiments compared to all treatments because it gave higher values in the most of growth parameters. Especially, the control treatment significantly increased nutrient uptake and nutrient concentration of the plant. It showed higher uptake of P, Mg, Ca, Cu, Zn, Ni by the plant in our experiment. The second-best treatment was RP + NemaTec that showed also higher plant height, biomass weight, nutrient uptake, and nutrient concentration. NemaTec belonged to the algae group. Therefore, the algae extract group was more effective than the fungal and bacterial groups in this experiment. However, some fungal and bacterial groups were also found that they could also contribute to some nutrient concentration and uptake by the plant (Table 5- 12 and Table 5-13).

Table 5- 13 Effect of combined application biostimulants and phosphate rock on growth different parameters of maize

Treatment _RP	Plant Height 2 weeks	Plant Height 5 weeks	Biomass Weight	Highest Nutrient Uptake by plant	Highest Nutrient Content in Plant
Contol (RP)				Fe, Ni	Fe, Ni
Proradix + RP					
RV42 + RP	***			Zn,Ca	
RhizoVitall 45 + RP					P, Ca
MUCI + RP					K
RV42 + MUCI + RP				K	
SuperFifty + RP					S
NemaTec + RP		***	***	P,Mg,Cu, Mn	
LamVita + RP				B,S	B
Biological Fertilizer OD + RP					Mg,Cu Zn, Mn
Trichoderma OMG + RP					

(***) = highest value within treatments

The application of MUCI in both treatments resulted in the lowest values in many growth parameters. However, the combined application of RP + MUCI shows the higher K concentration in plants after the combined application of RP + Trichoderma OMG. Moreover, the combined application of RP + RV 42 + MUCI showed the highest K uptake by the plant.

The highest Fe content was found at MUCI, RV 42 + MUCI and Control while the highest Fe uptake was also found at MUCI, RV 42 + MUCI, and Control. We assumed that the application of MUCI was effective in K and Fe content and uptake by the plant.

Table 5- 14 Effect of biostimulants application on different growth parameters of maize

Treatment_control	Plant Height		Biomass Weight	Highest Nutrient Uptake by Plant	Highest Nutrient Content in Plant
	2 weeks	5 weeks			
Control				P, Mg,Ca,Cu,Zn, Ni	P, Cu, Ni
Proradix	***		***	Mn,	Mn
RhizoVital 42 (RV42)					
RhizoVitall 45					Ca,Mg,Zn
MUCI					
RV42 + MUCI				B,	B
SuperFifty					
NemaTec		***		Fe,	Fe
LamVita				S,	S
Biological Fertilizer OD					
Trichoderma OMG				K,	K,

(***) = highest value within treatments

CHAPTER 6
6. DISCUSSION

Many scientific studies reported that biostimulants application have a positive effect on plant yield (García-Martínez et al. 2010). The yield of plant is usually determined as the amount of fruit obtained from one plant or plot. The yield increased by biostimulants depends on the type of biostimulant used, the dose, the method of application and the plant variety. Increased yield is often associated with improving the quality of vegetables or fruit. This is particularly important in organic farming, where chemical fertilizers cannot be used (Kocira et al. 2015 and Milić et al., 2018).

The main objective of our experiment is to investigate the effect of biostimulant on maize growth and yield and to find out the best biostimulants application. Therefore, plant height, aboveground biomass weight, nutrient concentration and nutrient uptake by the plant were measured to determine the maize growth and yield by bistimulants application. In our experiment, not all biostimulants treatments significantly effective as good as control (only soil) in only biostimulants application. Control (soil only) was the best treatment in only biostimulants application. However, when we compared only biostimulants application and combined application of RP + biostimulant, RP + biostimulants treatments showed higher values which were effective in many parameters. Therefore, biostimulants application would be effective with phosphate rock. Furthermore, the negative effect was determined at the treatments with the application of the biostimulant MUCI. However, some positive effects were recorded with RP + MUCI in K uptake, RP + RV 42 + MUCI in K content and the highest Fe content was found at MUCI, and RV 42 + MUCI. The soil application of MUCI had no beneficial effect on maize growth could be related due to inefficient P solubilisation. Therefore, P remained to be the most limiting nutrient (Mercl et al. 2018).

6.1 Plant height

Plant height was measured two times, two weeks after sowing and five weeks after sowing. According to our results, the highest plant height was found at both measurements in biostimulants applications. Therefore, in this case, biostimulants applications could be effective to stimulate plant growth and development. NemaTec, seaweed extracts treatment showed highest plant height at five weeks after sowing. The higher germination percentage and seedling vigour at low concentrations of seaweed extracts might be due to the presence of growth promoting substances such as auxins, gibberellins, phenyl acetic acid (Sivasankari et al., 2006) and other micronutrients (Layek et al. 2014). It has been also reported that application of seaweed both improved the growth of the crop and helped in increasing the number of functional nodules as compared to control due to presence of several cytokinins, which are found in brown algale extracts, including, trans-zeatin riboside, and its dihydro derivatives (Saravanan et al., 2003). PGPR helps plants directly or indirectly to increase plant growth-promoting attributes such as increase in seedling emergence, effective nodulation as well as nodule functioning, enhanced, increased indigenous plant hormones, root hair proliferation, root hair deformation and branching, early mineral and water uptake, promote the accumulation of carbohydrates and increasing the yield (Kishore, 2006). However, the negative effect of biostimulants on plant height was found at MUCI treatment. All of the biostimulants based on microorganisms (arbuscular mycorrhizal fungi + *Pseudomonas* sp. Strain 19Fv1T, arbuscular mycorrhizal fungi + *Pseudomonas fluorescens* C7, arbuscular mycorrhizal fungi + *Pseudomonas* sp. 19 Fv1T and *Pseudomonas fluorescens* C7) were tested in tomato cultivation. This study showed that the most effective result was found by biostimulants including arbuscular mycorrhizal fungi and *P. fluorescent* C7, which caused an increase in tomato mass (Bona et al. 2018). The positive effects of PGPF are found from the very early stage of crop development influencing germination and seedling growth. Different type of PGPF species differ significantly in their effect on seed germination and seedling growth (Yedidia et al., 2001). According this study, not all biostimulants are effective to every crop. There may be the different responses of biostimulants to different crops.

6.2 Aboveground biomass weight

Above-ground biomass (AGB) is one of the important indicators for effectively assessing crop growth and yield. It is also a vital ecological indicator for assessing the efficiency with which crops use light and store carbon in ecosystems (Zhu et al. 2019). In this case, the higher aboveground biomass weight was recorded with RP + biostimulants application. The highest treatment was NemaTec + RP. Therefore, NemaTec belonged to the seaweed extracts group was significantly higher in plant growth and development. Seaweed extracts perform as bio-stimulants mainly due to the presence of plant hormones. Mostly, auxins, cytokinins, gibberelins, abscisic acid and ethylene can be found. Auxins are needed for elongational growth of plant tissues and apical dominance, cell division, plant movements and plant aging. Cytokinins are important in cell division regulation affecting plant growth and rest period (Officer, 2014). One study has shown that the positive influence of biostimulants based on humic, fulvic, and carboxylic acids on the yielding of apricot fruits. In the control treatment, trees showed a yield of 12 kg fruit/tree. However, after the application of humic and fulvic acids together and carboxylic acids in a separate experiment, the yield of the trees increased to 21 kg of fruit/tree and 19 kg of fruit/tree, respectively. But this positive effect was showed only in the second year of using the biostimulant. During the first growing season, the control trees showed a higher yield than that of the trees that were treated with biostimulants containing humic and fulvic acids (Tarantino et al., 2018). According to this study, some biostimulants may need a certain time to be effective on crop growth and yield.

6.3 Nutrient concentration in aboveground biomass

Most designs for the plant analysis to determine nutrient status are based on the relationship between nutrient concentration and yield or growth of a plant or plant part. There are different ways to express concentration, but the most common are percent (%) and mg/kg (or part per million, or ppm). Percent is commonly used for the major nutrients – N, P, K, S, Mg and Ca – while ppm is used for the micronutrients (Fageria et al., 2011).

6.3.1 Macronutrients concentration

The highest P values were recorded for Trichoderma OMG followed by Control (soil only). For K content, the highest values were found for Trichoderma OMG and MUCI + RP. The highest Ca content was recorded for RhizoVital 45 and control (soil only). The maximum Mg content was found at RhizoVital 45 and control (soil only). LamVita, NemaTec and SuperFifty were highest in S content. Therefore, the highest nutrient concentration was found with biostimulants treatments, but control treatment also showed in higher nutrient concentration. According to this result, macronutrients concentration was also increased by RhizoVital 45 and MUCI + RP belonged to the bacterial group. Plant growth promoting bacteria (PGPB) have many benefits in agriculture by increasing crop productivity and nutrient content and suppressing the growth of pathogens. Interaction of beneficial plant-microbe based on genomics, transcriptomics, proteomics and metabolomic data of PGPB. The host plant will lead to enhance microbial inoculants for increasing crop yield and nutrient content. PGPB are described as a green technology due to reducing the use of chemical fertilizers thereby improving soil health (Ramakrishna et al., 2019).

6.3.2 Micronutrient concentration

MUCI, RV42 + MUCI and Control (RP only) were highest in Fe content. Control, RhizoVital 45, and RV 42 were recorded as the highest Cu content. RhizoVital 45, Control (soil only), and LamVita showed the highest Zn content. Proradix, MUCI and RV 42 were also highest in Mn content between two applications. The highest B content was found at RV42 + MUCI, RhizoVital 45 and LamVita + RP between two applications. Control (Soil + RP only) was highest in Ni content followed by Control and MUCI between two applications. In this case, biostimulants treatments were higher nutrient concentration than control. Biostimulants can be used in the different form of application such as soil preparations (powders, granules, or solutions added to the soil) or as liquid foliar application products (Kocira et al., 2018). While biostimulants containing humic substances and nitrogen compounds can be used directly onto the soil, various types of extracts from plants and seaweed are used in the form of foliar applications. This study reported that the soil application of the biostimulant was not as effective as the foliar application in maize cultivation. The foliar application of a biostimulant extracted from sewage sludge resulted in an increase of macro- and micronutrients level in the leaves of maize. For the above reason, biostimulants application could show different results by using different methods of biostimulants application. According to our study, even soil biostimulants application showed better results in nutrient concentration than control. However, the yield of strawberry was significantly increased after using biostimulants containing herbal and marine plant extracts. In this study, soil biostimulants application are a source of nitrogen compounds. Moreover, this study reported that foliar biostimulant application was not effective as good as biostimulants added to the soil. Soil biostimulants application caused a significant increase in the amount of fruit and also promoted the condition of the plants (Filipczak et al., 2016). Thus, effectiveness of biostimulants on crop growth and yield may also depend on crops and method of applications.

6.4 Nutrient uptake by plant

6.4.1 Macro nutrients uptake by plant

The use of AMF can significantly increase the phosphorus concentration in both root and shoot systems (Al Hmoud and Al-Momany, 2017). Mycorrhizal association can increase phosphorus supply to the infected roots of host plants under phosphorus-limited soil conditions (Bucher, 2007). In maize and other crops, the most widely recognized contribution of AM fungi to host-plant nutrition involves their ability to extract P from outside the P depletion zone around plant roots (Liu et al., 2000). However, the highest P and Mg uptake were recorded with RP + NemaTec. The highest P uptake could be related with the addition of phosphate rock. For many years, seaweed has been applied as a fertilizer in coastal regions (Craigie, 2011). Some plant nutrients can be found in seaweed extracts. When applied to plants grown in a nutrient-deficient medium soil, the simple availability of these nutrients may improve growth and nutrient uptake (Khan et al., 2009). Mostly, RP + biostimulants treatments were more effective than only biostimulants treatments on uptake of macronutrients (P, K, Mg, and S). In many cases, negative results were obtained by the application of MUCI in our experiment. However, the highest K uptake was found at RP + RV42 + MUCI. The highest Ca uptake was found at Control (soil only). Therefore, bisotimulants application was not effective in Ca uptake. The highest S uptake was found at RP + LamVita. Soil microorganism are essential and important to soil and nutrient availability. However, their activities are influenced by proper soil environmental conditions such as soil temperature, organic matter content and soil pH, etc. For example, soil temperature is important for the activity of soil microorganisms. The rate of reaction of the soil is affected by soil temperature. Higher temperature increases the activity of soil microorganism. At high soil temperature, the rate of decomposition and degradation of soil organic matter is faster and the release of nutrients into soil also faster. The presence of soil organic matter is also important for soil microbial activity. Soil organisms feed on organic matter; without organic matter, living become unbearable to them. At the time, leads to their detrimental effects on crops. The soil pH is one of the important factors to increase the uptake of most macro and micronutrients by plants from the soil. Optimal soil pH for microorganism ranges from 6 – 8 (AbdulQuadri, 2017). Therefore, one of the suitable methods to increase the availability of soil nutrients to plant is the adjustment of soil pH by application of Sulphur containing fertilizer like gypsum or lime to the soil (Kopecky, 2014).

Many studies have shown the state confusion in the field of biostimulants (Traon et al., 2014). Therefore, biostimulant market is not based on science or efficacy. According to some studies, research on several biostimulant products has shown them to be ineffective or to contain inactive, unstable or inconsistent properties with several showing negative effects compared when contrasted with well-designed controls. As our result, we found that some biostimulants have the specific ability for specific nutrient. Therefore, we cannot assume that not all biostimulants good for every single nutrient uptake.

6.4.2 Micronutrients uptake by plant

The higher uptake of Fe was recorded for MUCI while the highest one is control (RP only). Although MUCI application showed negative effects in many cases, it also was recorded as the highest Fe uptake by maize. Fe is also abundant in soils but mostly in the insoluble Fe^{+III} oxide form, such as hematite, goethite, and ferrihydrite in calcareous soils, Fe is mostly unavailable for plant uptake because the alkaline conditions render the Fe less soluble. Certain bacteria produce siderophores, which chelate Fe, making it more soluble. One study showed that maize and sunflower have better Fe uptake in nonsterile calcareous soils than in their sterile counterparts (Masalha et al., 2000). The higher uptake of Cu was recorded for control (soil only) and the highest Zn were recorded for control (soil only). Control (RP only) was recorded as the highest Ni in both applications. Therefore, biostimulants application was less effective for these nutrient uptakes. However, Mn uptake was increased by Proradix application and control. Manganese may not be deficient under dry climates and sometimes it can be found at high amounts. Soil biological activities can also influence on Mn availability in the soil especially by affecting plant growth and hence plant root exudates (Dutta and Podile 2010; Miransari, 2011d). There was no significant difference in B uptake by aboveground dry biomass. But the highest uptake was recorded for RP + LamVita and RV 42 + MUCI. The vital point to use of soil microbes for biofertilization is selecting the right combination of microbes (the enhancing interactions between the microbial strains), determining their inoculating potential, their persistence in the soil, their survival under stress, etc. Different microbial species and strains may respond differently under different conditions, determining their efficiency and hence their related use (Miransari, 2013).

CHAPTER 7

7. CONCLUSION

According to the literature, the use of biostimulants on a commercial scale would limit the amount of mineral fertilizers introduced into the environment that will lead to reduce the pollution of soils, water, and air. These facts are very important for global warming, safety environment and sustainable production for the growing population in the world. Many factors can influence the effectiveness of biostimulants, from the raw material processing to their response to the plant varieties, application method, and soil-climatic conditions. The main advantages of biostimulants are positive impacts on crop quality and performance, no negative or harmful impact on people, animals, or the environment, increased biodiversity of beneficial microorganisms, and improvement of soil properties.

The screening pot experiment was set up to investigate the effect of biostimulants on maize growth and yield. The soil was typical, e.g. with low content of P. Maize was grown in a greenhouse under treatments consisting of two types of fertilizer application, biostimulants only and combined application of rock phosphate (RP) with biostimulants. Proradix, RhizoVital 42 (RV42), RhizoVital 45, MUCI, RV42 + MUCI, SuperFifty, NemaTec, LamVita, Biological Fertilizer OD and Trichoderma OMG were used as biostimulants to investigate the effect on maize growth and nutrient contents and uptake in both applications.

According to our results, positive effect of biostimulants did not occur in single biostimulants application. Control (soil only) treatment was the best one here, because it was recorded as a higher nutrient concentration in aboveground biomass and higher nutrient uptake treatment. However, some positive effect was obtained in the combined application of RP + biostimulants treatments. NemaTec treatment, which belongs to the seaweed extracts group, was the effective one within the combined application of RP + biostimulants application. RP + NemaTec provided better results in plant height, biomass weight, and uptake of P, Mg, Cu, Mn when compared to all other biostimulants application in our experiment. It also demonstrated that it could be able to increase plant height, biomass weight and uptake of P, Mg, Cu, Mn than control (soil only). Moreover, mostly, RP + biostimulants treatments which were effective in the experiment presented higher values at plant height, nutrient concentration, and nutrient uptake compared to control (soil with RP only) which was the best treatment in single biostimulants application. Therefore, NemaTec treatment was an effective biostimulant treatment by using together with phosphate rock at the vegetative growth stage of maize. The negative effects of biostimulant were recorded with MUCI because it was not effective in most of the investigated parameters. However, some positive effects were recorded with MUCI + RP in K uptake, MUCI + RP in K content and the higher Fe content was found at MUCI. According to the above-mentioned reasons it is clear that some positive effects of biostimulants were found in the application of biostimulants with RP, while not all biostimulant treatments were significantly effective in biostimulants only application.

To better understand the effect of biostimulants on maize growth, we have to proceed more research about their application in different soils and specific maize varieties. Our experiment only focused on short period of vegetative growth. We need to investigate also the long-term effect of biostimulants on maize growth and changes of soil physical and chemical properties in this local soil because some biostimulants strains and plant extracts do not show their positive effects in such a short period. To get more reliable reasons for using biostimulants, different method of applications (foliar, local soil application or seed treatment), different doses and different varieties are also should be examined.

The nature of their positive influence on crop and soil properties is not completely understood. Thus, their mechanisms of action are still a challenge and need to be recognized in some cases. Not all biostimulants would be effective in each crops, locations, soils, and climates. Therefore, specific biostimulants should be prepared on the specific variety, methods of application, location, and environment.

8. REFERENCES

AbdulQuadri, A. (2017). Importance of all soil microorganisms in agriculture. Retrieved from: https://justagric.com/soil-microorganisms/

Adani, F., Genevini, P., Zaccheo, P., & Zocchi, G. (1998). The effect of commercial humic acid on tomato plant growth and mineral nutrition. *Journal of Plant Nutrition*, *21*(3), 561–575.

Alam, M., Khaliq, A., Sattar, A., Shukla, R. S., Anwar, M., & Dharni, S. (2011). Synergistic effect of arbuscular mycorrhizal fungi and Bacillus subtilis on the biomass and essential oil yield of rose-scented geranium (Pelargonium graveolens). *Archives of Agronomy and Soil Science*, *57*(8), 889–898.

Alavi, P., Starcher, M. R., Zachow, C., Müller, H., & Berg, G. (2013). Root-microbe systems: The effect and mode of interaction of stress protecting agent (SPA) Stenotrophomonas rhizophila DSM14405T. *Frontiers in Plant Science*, *4*(MAY), 1–10.

Albrecht, U. (2019). *Plant Biostimulants : Definition and Overview of Categories and Effects 1*. 1–4.

Al-Hmoud G., & Al-Momany, A. (2017). Effect of Four Mycorrhizal Products on Squash Plant Growth and its Effect on Physiological Plant Elements. *Advances in Crop Science and Technology*, *05*(01), 1–6.

Amin, M., Ahmad, R., Ali, A., Hussain, I., Mahmood, R., Aslam, M., & Lee, D. J. (2018). Influence of Silicon Fertilization on Maize Performance Under Limited Water Supply. *Silicon*, *10*(2), 177–183.

Anonym (2020) European biostimulants industry council. Retrieved from: http://www. biostimulants.eu/

Artyszak, A. (2018). Effect of silicon fertilization on crop yield quantity and quality—A literature review in Europe. *Plants*, *7*(3).

Atiyeh, R. M., Lee, S., Edwards, C. A., Arancon, N. Q., & Metzger, J. D. (2002). The influence of humic acids derived from earthworm-processed organic wastes on plant growth. *Bioresource Technology*, *84*(1), 7–14.

Augé, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, *11*(1), 3–42.

Balan, V., & Verestiuc, L. (2014). Strategies to improve chitosan hemocompatibility: A review. *European Polymer Journal*, *53*(1), 171–188.

Bashan, Y., & De-Bashan, L. E. (2005). Plant growth-promoting. *Encyclopedia of soils in the environment*, *1*, 103-115.

Bashan, Y., & Holguin, G. (1998). Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry*, *30*(8–9), 1225–1228.

Battacharyya, D., Babgohari, M. Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 39–48.

Begum, M., Bordoloi, B. C., Singha, D. D., & Ojha, N. J. (2018). Role of seaweed extract on growth, yield and quality of some agricultural crops: A review. Agricultural Reviews, 39(of), 321–326.

Bidegain, R. A., Kaemmerer, M., Guiresse, M., Hafidi, M., Rey, F., Morard, P., & Revel, J. C. (2000). Effects of humic substances from composted or chemically decomposed poplar sawdust on mineral nutrition of ryegrass. The Journal of Agricultural Science, 134(3), 259-267.

Bona, E., Todeschini, V., Cantamessa, S., Cesaro, P., Copetta, A., Lingua, G., … Massa, N. (2018). Combined bacterial and mycorrhizal inocula improve tomato quality at reduced fertilization. *Scientia Horticulturae*, *234*(July), 160–165.

Bucher, M. (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytologist*, *173*(1), 11–26.

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., & Ferrante, A. (2015). Biostimulants and crop responses: A review. *Biological Agriculture and Horticulture*, *31*(1), 1–17.

Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant and Soil*, *383*(1–2), 3–41.

Chandini, Kumar, R., Kumar, R., & Prakash, O. (2019). The Impact of Chemical Fertilizers on our Environment and Ecosystem. *Research Trends in Environmental Sciences*, (February), 69.

Chen, J.-H. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility Jen-Hshuan Chen ? Department of Soil and Environmental Sciences, National Chung Hsing University, Taiwan, R.O.C. *International Workshop on Sustained Management*, (October), 1–11.

Chen, S., Zhao, H., Zou, C., Li, Y., Chen, Y., Wang, Z., … Ahammed, G. J. (2017). Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. *Frontiers in Microbiology*, *8*(DEC), 1–11.

Craigie, J. S. (2011). Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, *23*(3), 371–393.

Crouch, I. J., Beckett, R. P., & van Staden, J. (1990). Effect of seaweed concentrate on the growth and mineral nutrition of nutrient-stressed lettuce. *Journal of Applied Phycology*, *2*(3), 269–272.

du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3–14.

Dursun, A., Güvenç, İ., & Turan, M. (1999). Improved Crop Quality by Nutrient Management. Macro and Micro Nutrient Contents of Tomato and Eggplant Seedlings and Their Effects on Seedling Growth in Relation to Humic Acid Application. (Anaç, D. and Martin-Prevel, P., Eds). Kluwer Academic Publishers, Dordrecht, Boston, London.

Epstein, E. (1994). The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(1), 11–17.

Escudero, N., Lopez-Moya, F., Ghahremani, Z., Zavala-Gonzalez, E. A., Alaguero-Cordovilla, A., Ros-Ibañez, C., … Lopez-Llorca, L. V. (2017). Chitosan Increases Tomato Root Colonization by Pochonia chlamydosporia and Their Combination Reduces Root-Knot Nematode Damage. *Frontiers in Plant Science*, *8*(September), 1–10.

Estavillo, J. M., Rodriguez, M., Domingo, M., Muñoz-Rueda, A., & Gonzalez-Murua, C. (1994). Denitrification losses from a natural grassland in the Basque Country under organic and inorganic fertilization. *Plant and Soil*, *162*(1), 19–29.

Eyheraguibel, B., Silvestre, J., & Morard, P. (2008). Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Bioresource Technology*, *99*(10), 4206–4212.

Fageria, N. K., Baligar, V. C., & Jones, C. A. (2011). *Growth and Mineral Nutrition of Feild Crops*. 1–574.

Filipczak, J., Zurawicz, E., & Sas-Paszt, L. (2016). Wpływ wybranych biostymulatorów na wzrost i plonowanie roślin truskawki 'Elkat'. *Zeszyty Naukowe Instytutu Ogrodnictwa*, *24*.

Fornes, F., Sánchez-Perales, M., & Guardiola, J. (2002). Effect of a Seaweed Extract on the Productivity of "de Nules" Clementine Mandarin and Navelina Orange. *Botanica Marina - BOT MAR*, *45*, 486–489.

Gamalero, E., & Glick, B. R. (2011). Bacteria in Agrobiology: Plant Nutrient Management. In *Bacteria in Agrobiology: Plant Nutrient Management*.

García-Martínez, A. M., Díaz, A., Tejada, M., Bautista, J., Rodríguez, B., Santa María, C., … Parrado, J. (2010). Enzymatic production of an organic soil biostimulant from wheat-condensed distiller solubles: Effects on soil biochemistry and biodiversity. *Process Biochemistry*, *45*(7), 1127–1133.

García-Mina, J. M., Antolín, M. C., & Sanchez-Diaz, M. (2004). Metal-humic complexes and plant micronutrient uptake: A study based on different plant species cultivated in diverse soil types. *Plant and Soil*, *258*(1–2), 57–68.

García, A. C., Olaetxea, M., Santos, L. A., Mora, V., Baigorri, R., Fuentes, M., … Garcia-Mina, J. M. (2016). Involvement of Hormone-and ROS-Signaling Pathways in the Beneficial Action of Humic Substances on Plants Growing under Normal and Stressing Conditions. *BioMed Research International*, *2016*.

Guan, Y. J., Hu, J., Wang, X. J., & Shao, C. X. (2009). Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *Journal of Zhejiang University: Science B*, *10*(6), 427–433.

Guler, N. S., Pehlivan, N., Karaoglu, S. A., Guzel, S., & Bozdeveci, A. (2016). Trichoderma atroviride ID20G inoculation ameliorates drought stress-induced damages by improving antioxidant defence in maize seedlings. *Acta Physiologiae Plantarum*, *38*(6).

Halifu, S., Deng, X., Song, X., & Song, R. (2019). Effects of Two Trichoderma Strains on Plant Growth, Rhizosphere Soil Nutrients, and Fungal Community of Pinus sylvestris var. mongolica Annual Seedlings. *Forests*, *10*(9), 758.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The Use of Biostimulants for Enhancing Nutrient Uptake. In *Advances in Agronomy* (Vol. 130).

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology*, *60*(4), 579–598.

Hossain, M. M., Sultana, F., Miyazawa, M., & Hyakumachi, M. (2014). The plant growth-promoting fungus Penicillium spp. GP15-1 enhances growth and confers protection against damping-off and anthracnose in the cucumber. *Journal of Oleo Science*, *63*(4), 391–400.

Houba V. J. G., Novozamsky I., Huybreghts A. W. M., Vad Der Lee J.J. (1986) Comparison of soil extraction by 0,01 M $CaCl_2$, by EUF and by some conventional extraction procedures. Plant and Soil 96:433–437

ISO 10390 (1994) Soil quality–Determination of pH, 1994, 1:1–17.

Jiang, Y., & Li, Y. (2001). Effects of chitosan coating on postharvest life and quality of longan fruit. *Food Chemistry*, *73*(2), 139–143.

Kahlon, S. S., & Malhotra, S. (1986). Production of gibberellic acid by fungal mycelium immobilized in sodium alginate. *Enzyme and Microbial Technology*, *8*(10), 613–616.

Katiyar, D., Hemantaranjan, A., & Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology*, *20*(1).

Kayama, M., & Yamanaka, T. (2014). Growth characteristics of ectomycorrhizal seedlings of Quercus glauca, Quercus salicina, and Castanopsis cuspidata planted on acidic soil. *Trees - Structure and Function*, *28*(2), 569–583.

Khalil, S. E. (2016). Alleviating salt stress in Thymus capitatus plant using plant growth-promoting bacteria (PGPR). *International Journal of ChemTech Research*, *9*(9), 140–155.

Khan, A. L., Shinwari, Z. K., Kim, Y. H., Waqas, M., Hamayun, M., Kamran, M., & Lee, I. J. (2012). Role of endophyte chaetomium globosum lk4 in growth of capsicum annuum by producion of gibberellins and indole acetic acid. *Pakistan Journal of Botany*, *44*(5), 1601–1607.

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., … Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation*, *28*(4), 386–399.

King, K. W., & Torbert, H. A. (2007). Nitrate and ammonium losses from surface-applied organic and inorganic fertilizers. *Journal of Agricultural Science*, *145*(4), 385–393.

Kishore, G. K. (2006). *Plant growth-promoting rhizobacteria*. (Table 1), 195–196.

Kocira, A., Świeca, M., Kocira, S., Złotek, U., & Jakubczyk, A. (2018). Enhancement of yield, nutritional and nutraceutical properties of two common bean cultivars following the application of seaweed extract (Ecklonia maxima). *Saudi Journal of Biological Sciences*, *25*(3), 563–571.

Kocira, S., Sujak, A., Kocira, A., Wójtowicz, A., & Oniszczuk, A. (2015). Effect of Fylloton Application on Photosynthetic Activity of Moldavian Dragonhead (Dracocephalum moldavica L.). *Agriculture and Agricultural Science Procedia*, *7*(January 2016), 108–112.

Kopecky, M. (2014, June 2). When to Use Lime, Gypsum and Elemental Sulfur. Retrieved from https://onpasture.com/2014/06/02/when-to-use-lime-gypsum-and-elemental-sulfur/

Kumar, M. R., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical reviews*, *104*(12), 6017-6084.

Kumar, R., Kumar, R., & Prakash, O. (2019). Chapter-5 The Impact of Chemical Fertilizers on Our Environment and Ecosystem. *Chief Editor*, *35*, 69.

Layek, J., Ramkrushna, G. I., Das, A., Ghosh, A., Krishnappa, R., Panwar, A. S., ... & Mawlong, B. (2014). Seaweed sap as organic bio-stimulant for rice and maize production. *Research bulletin*, *82*.

Lee, Y. S., Kim, Y. H., & Kim, S. B. (2005). Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. *HortScience*, *40*(5), 1333–1335.

Lenka, S., Rajendiran, S., Coumar, V., & Dotaniya, M. L. (2016). Impact of Fertilizers use on Environmental Quality. *National Seminar on Environmental Concern for Fertilizer Use in Future at Bidhan Chandra Krishi Viswavidyalaya, Kalyani on February*, (26).

Liu, A., Hamel, C., Hamilton, R. I., Ma, B. L., & Smith, D. L. (2000). Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (Zea mays L.) grown in soil at different P and micronutrient levels. *Mycorrhiza*, *9*(6), 331–336.

Ma, G., Yang, D., Zhou, Y., Xiao, M., Kennedy, J. F., & Nie, J. (2008). Preparation and characterization of water-soluble N-alkylated chitosan. *Carbohydrate Polymers*, *74*(1), 121–126.

Ma, J. F., & Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, *11*(8), 392–397.

Ma, L.-J., Li, Y.-Y., Wang, L.-L., Li, X.-M., Liu, T., & Bu,. (2014). Germination and Physiological Response of Wheat (Triticum aestivum) to Pre-soaking with Oligochitosan. *International Journal of Agriculture and Biology*, *16*, 766–770.

Maček, I. (2017). Arbuscular mycorrhizal fungi in hypoxic environments. In *Mycorrhiza - Function, Diversity, State of the Art: Fourth Edition*.

Mader, P., Száková, Milholová, J. D. Classical dry ashing of biological and agricultural materials. Part II. Losses of analytes due to their retention in an insoluble residue. Analusis, 26 (1998), pp. 121–129

Maghsoudi, K., Emam, Y., & Ashraf, M. (2016). Foliar application of silicon at different growth stages alters growth and yield of selected wheat cultivars. *Journal of Plant Nutrition*, *39*(8), 1194–1203.

Masalha, J., Kosegarten, H., Elmaci, Ö., & Mengel, K. (2000). The central role of microbial activity for iron acquisition in maize and sunflower. *Biology and Fertility of Soils*, *30*(5–6), 433–439.

Mehlich A. (1984): Mehlich 3 soil test extraction: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis, 15: 1409–1416.

Mercl, F., Tejnecký, V., Ságová-Marečková, M., Dietel, K., Kopecký, J., Břendová, K., … Tlustoš, P. (2018). Co-application of wood ash and Paenibacillus mucilaginosus to soil: the effect on maize nutritional status, root exudation and composition of soil solution. *Plant and Soil*, *428*(1–2), 105–122.

Milić, B., Tarlanović, J., Keserović, Z., Magazin, N., Miodragović, M., & Popara, G. (2018). Bioregulators can improve fruit size, yield and plant growth of northern highbush blueberry (Vaccinium corymbosum L.). *Scientia Horticulturae*, *235*(March), 214–220.

Miransari, M. (2013). Soil microbes and the availability of soil nutrients. *Acta Physiologiae Plantarum*, *35*(11), 3075–3084.

Murphy, J. G., Rafferty, S. M., & Cassells, A. C. (2000). Stimulation of wild strawberry (Fragaria vesca) arbuscular mycorrhizas by addition of shellfish waste to the growth substrate: Interaction between mycorrhization, substrate amendment and susceptibility to red core (Phytophthora fragariae). *Applied Soil Ecology*, *15*(2), 153–158.

Murray, J. D. (2011). Invasion by invitation: Rhizobial infection in legumes. *Molecular Plant-Microbe Interactions*, *24*(6), 631–639.

O'Herlihy, E. A., Duffy, E. M., & Cassells, A. C. (2003). The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from microplants. *Folia Geobotanica*, *38*(2), 201–207.

O Duke, S., & B Powles, S. (2008). Glyphosate: a once-in-a-century herbicide. *Pest Management Science*, *63*(11), 1100–1106.

Officer, Q. A. (2014). JOB PURPOSE : Responsible for assisting the Director Quality Assurance in the development , implementation and promotion of quality assurance systems , structures in all University Academic operations , non-Academic , Administrative and other support serv. (7), 639–641.

Ohta, K., Atarashi, H., Shimatani, Y., Matsumoto, S., Asao, T., & Hosoki, T. (2000). Effects of chitosan with or without nitrogen treatments on seedling growth in Eustoma grandiflorum (Raf.) Shinn. Cv. Kairyou Wakamurasaki. *Journal of the Japanese Society for Horticultural Science*, *69*(1), 63-65.

Okoth, S. A., Otadoh, J. A., & Ochanda, J. O. (2011). Improved seedling emergence and growth of maize and beans by Trichoderma harziunum. *Tropical and Subtropical Agroecosystems*, *13*(1), 65–71.

Oldroyd, G. E. D., Murray, J. D., Poole, P. S., & Downie, J. A. (2011). The Rules of Engagement in the Legume-Rhizobial Symbiosis. *Annual Review of Genetics*, *45*(1), 119–144.

Oliveira, A. L. M., Stoffels, M., Schmid, M., Reis, V. M., Baldani, J. I., & Hartmann, A. (2009). Colonization of sugarcane plantlets by mixed inoculations with diazotrophic bacteria. *European Journal of Soil Biology*, *45*(1), 106–113.

Orientador, J. R., Ramos, L., & Barbosa, S. (2014). *Departamento de Física Geral Projeto de Mestrado*. *133*(September), 1–13.

Ortas, I. (2012). The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. *Field Crops Research*, *125*, 35–48.

Pal, A., Dwivedi, S. K., Maurya, P. K., & Kanwar, P. (2015). Effect of seaweed saps on growth, yield, nutrient uptake and economic improvement of maize (sweet corn).

Journal of Applied and Natural Science, *7*(2), 970–975.

Parray, J. A., Jan, S., Kamili, A. N., Qadri, R. A., Egamberdieva, D., & Ahmad, P. (2016). Current Perspectives on Plant Growth-Promoting Rhizobacteria. *Journal of Plant Growth Regulation*, *35*(3), 877–902.

Pérez-Montaño, F., Alías-Villegas, C., Bellogín, R. A., Del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., … Cubo, T. (2014). Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiological Research*, *169*(5–6), 325–336.

Qavami, N., Naghdi Badi, H., Labbafi, M., Mehregan, M., Tavakoli, M., & Mehrafarin, A. (2017). Overview on Chitosan as a valuable ingredient and biostimulant in pharmaceutical industries and agricultural products. *Trakia Journal of Science*, *15*(1), 83–91.

Qiuping, Z., & Wenshui, X. (2007). Effect of 1-methylcyclopropene and/or chitosan coating treatments on storage life and quality maintenance of Indian jujube fruit. *LWT - Food Science and Technology*, *40*(3), 404–411.

Ramakrishna, W., Yadav, R., & Li, K. (2019). Plant growth promoting bacteria in agriculture: Two sides of a coin. *Applied Soil Ecology*, *138*(March), 10–18.

Rathore, S. S., Chaudhary, D. R., Boricha, G. N., Ghosh, A., Bhatt, B. P., Zodape, S. T., & Patolia, J. S. (2009). Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (Glycine max) under rainfed conditions. *South African Journal of Botany*, *75*(2), 351–355.

Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S. L., Morton, J. B., & Walker, C. (2013). An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza*, *23*(7), 515–531.

Rodríguez, H., & Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, *17*(4–5), 319–339.

Russo, R. O., & Berlyn, G. P. (1991). The Use of Organic Biostimulants to Help Low Input Sustainable Agriculture. *Journal of Sustainable Agriculture*, *1*(2), 19–42.

Sadhana, B. (2014). Review Article Arbuscular Mycorrhizal Fungi (AMF) as a Biofertilizer- a Review. *Int.J.Curr.Microbiol.App.Sci*, *3*(4), 384–400.

Saravanan, S., Thamburaj, S., Veeraragavathatham, D., & Subbiah, A. (2003). Effect of seaweed extract and chlormequat on growth and fruit yield of tomato (Lycopersicon esculentum Mill.). *Indian Journal of Agricultural Research*, *37*(2), 79-87.

Savci, S. (2012). Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia*, *1*(January), 287–292.

Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). Food applications of chitin and chitosans. *Trends in Food Science and Technology*, *10*(2), 37–51.

Sharif, M., Khattak, R. A., & Sarir, M. S. (2002). Effect of different levels of lignitic coal derived humic acid on growth of maize plants. *Communications in Soil Science and Plant Analysis*, *33*(19–20), 3567–3580.

Sharma, A., Johri, B. N., Sharma, A. K., & Glick, B. R. (2003). Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (Vigna radiata L. Wilzeck). *Soil Biology and Biochemistry*, *35*(7), 887–894.

Shwethakumari, U., & Prakash, N. B. (2018). Effect of foliar application of silicic acid on soybean yield and seed quality under field conditions. *Journal of the Indian Society of Soil Science*, *66*(4), 406–414.

Sivakumar, D., Sultanbawa, Y., Ranasingh, N., Kumara, P., & Wijesundera, R. L. C. (2005). Effect of the combined application of chitosan and carbonate salts on the incidence of anthracnose and on the quality of papaya during storage. *Journal of Horticultural Science and Biotechnology*, *80*(4), 447–452.

Sivasankari, S., Venkatesalu, V., Anantharaj, M., & Chandrasekaran, M. (2006). Effect of seaweed extracts on the growth and biochemical constituents of Vigna sinensis. *Bioresource Technology*, *97*(14), 1745–1751.

Smith, S. E., Jakobsen, I., Grønlund, M., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology*, *156*(3), 1050–1057.

Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., … Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, *108*(5), 1028–1046.

Spiegel, Y., Kafkafi, U., & Pressman, E. (1988). Evaluation of a protein-chitin derivative of crustacean shells as a slow-release nitrogen fertilizer on Chinese cabbage. *Journal of*

Horticultural Science, *63*(4), 621–627.

Sulieman, S., & Tran, L. S. P. (2014). Symbiotic nitrogen fixation in legume nodules: Metabolism and regulatory mechanisms. *International Journal of Molecular Sciences*, *15*(11), 19389–19393.

Sun, Z., Song, J., Xin, X., Xie, X., & Zhao, B. (2018). Arbuscular mycorrhizal fungal 14-3-3 proteins are involved in arbuscule formation and responses to abiotic stresses during AM symbiosis. *Frontiers in Microbiology*, *9*(MAR), 1–17.

Szczepanek, M., & Wilczewski, E. (2016). Maize response to soil-applied humic substances and foliar fertilization with potassium. *Journal of Animal and Plant Sciences*, *26*(5), 1298–1303.

Tarantino, A., Lops, F., Disciglio, G., & Lopriore, G. (2018). Effects of plant biostimulants on fruit set, growth, yield and fruit quality attributes of 'Orange rubis®' apricot (Prunus armeniaca L.) cultivar in two consecutive years. *Scientia Horticulturae*, *239*, 26–34.

Terry, L. A., & Joyce, D. C. (2004). Elicitors of induced disease resistance in postharvest horticultural crops: A brief review. *Postharvest Biology and Technology*, *32*(1), 1–13.

Thirumaran, G., Arumugam, M., Arumugam, R., & Anantharaman, P. (2009). Effect of Seaweed Liquid Fertilizer on Growth and Pigment Concentration of Abelmoschus esculentus (l) medikus. *American-Eurasian Journal of Agronomy*, *2*(2), 57–66.

Traon, D., Amat, L., Zotz, F., & du Jardin, P. (2014). *A Legal Framework for Plant Biostimulants and Agronomic Fertiliser Additives in the EU*. (January), 133.

Turan, M., & Köse, C. (2004). Seaweed extracts improve copper uptake of grapevine. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *54*(4), 213–220.

Vamerali, T., Bandiera, M., & Mosca, G. (2010). Field crops for phytoremediation of metal-contaminated land. A review. *Environmental Chemistry Letters*, *8*(1), 1–17.

Varma, A., Prasad, R., & Tuteja, N. (Eds.). (2017). *Mycorrhiza-function, diversity, state of the art*. Berlin: Springer International Publishing.

Venkataraman Kumar, V., & Mohan, V. R. (1997). Effect of seaweed liquid fertiliser on black gram. *Phykos*, *36*(2), 43-47.

Xia, C., Li, N., Zhang, X., Feng, Y., Christensen, M. J., & Nan, Z. (2016). An Epichloë endophyte improves photosynthetic ability and dry matter production of its host Achnatherum inebrians infected by Blumeria graminis under various soil water conditions. *Fungal Ecology*, *22*(August), 26–34.

Yedidia, I., Srivastva, A. K., Kapulnik, Y., & Chet, I. (2001). Effect of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants. *Plant and Soil*, *235*(2), 235–242.

Yen, M. T., & Mau, J. L. (2007). Selected physical properties of chitin prepared from shiitake stipes. *LWT - Food Science and Technology*, *40*(3), 558–563.

Zandonadi, D. B., Santos, M. P., Busato, J. G., Peres, L. E. P., & Façanha, A. R. (2013). Plant physiology as affected by humified organic matter. *Theoretical and Experimental Plant Physiology*, *25*(1), 13–25.

Zandonadi, D. B., Santos, M. P., Caixeta, L. S., Marinho, E. B., Peres, L. E. P., & Façanha, A. R. (2016). Plant proton pumps as markers of biostimulant action. *Scientia Agricola*, *73*(1), 24–28.

Zeng, D., & Luo, X. (2012). Physiological Effects of Chitosan Coating on Wheat Growth and Activities of Protective Enzyme with Drought Tolerance. *Open Journal of Soil Science*, *02*(03), 282–288.

Zhu, Y., Zhao, C., Yang, H., Yang, G., Han, L., Li, Z., … Lei, L. (2019). Estimation of maize above-ground biomass based on stem-leaf separation strategy integrated with LiDAR and optical remote sensing data. *PeerJ*, *2019*(9), 1–30.

Zou, P., Yang, X., Wang, J., Li, Y., Yu, H., Zhang, Y., & Liu, G. (2016). Advances in characterisation and biological activities of chitosan and chitosan oligosaccharides. *Food Chemistry*, *190*(12), 1174–1181.

9. ENCLOSURES

9.1 List of abbreviation

AGB	Above-ground biomass
AMF	Arbuscular mycorrhizal fungi
AMV	Alfalfa mosaic virus
EBIC	European bio-stimulants industry council
GA	Gibberellic acid
GHG	Greenhouse gases
HS	Humic substances
INM	Integrated nutrient management
PGPB	Plant growth promoting bacteria
PGPF	Plant growth-promoting fungi
PHS	Protein hydrolysates
RP	rock phosphate
RV 45	RhizoVital 45
RV42	RhizoVital 42
SE	Seaweed extracts
SLF	Seaweed liquid fertilizers
WHC	Water holding capacity

9.2 List of tables

9.3 List of figures

9.4 Appendix

Appendix- i Measurement of Plant height and above-ground biomass between Control and Rock-Phosphate

Sr. no	Treatment		Plant Height: 2 weeks after sowing	Plant Height: 5 weeks after sowing	Above-ground Biomass (g)
1	Control	Control	22.75	69.13	1.75
		RP*	23.44	68.25	1.84
2	Proradix	Control	24.13	69.50	1.88
		RP	22.88	70.00	1.99
3	RhizoVital 42 (RV42)	Control	22.38	66.13	1.62
		RP	25.19	72.75	2.09
4	RhizoVitall 45	Control	22.88	69.63	1.56
		RP	24.25	71.13	1.93
5	MUCI	Control	18.38	62.75	1.38
		RP	21.00	68.38	1.63
6	RV42 + MUCI	Control	20.13	64.38	1.43
		RP	19.94	67.00	1.81
7	SuperFifty	Control	22.63	66.63	1.54
		RP	22.56	66.25	1.78
8	NemaTec	Control	22.38	71.00	1.68
		RP	24.13	73.75	2.19
9	LamVita	Control	22.75	67.25	1.57
		RP	22.63	68.88	2.18
10	Biological Fertilizer OD	Control	23.94	67.13	1.78
		RP	22.75	68.50	1.97
11	Trichoderma OMG	Control	22.31	66.13	1.69
		RP	22.81	70.38	2.04

*RP~ Rock Phosphate

Remark - Mean value for each parameter is used as basis comparison value.

Appendix- ii Measurement of element content in the aboveground dry biomass between Control and Rock-Phosphate

Sr. no	Treatment		Elements content in the aboveground dry biomass (mg/kg)				
			P	K	Ca	Mg	S
1	Control	Control	1767.09	12173.08	8275.83	3174.84	569.55
		RP*	1562.93	11325.99	6074.16	2662.95	568.37
2	Proradix	Control	1578.99	11909.60	6704.34	2624.64	504.48
		RP	1549.41	9048.43	6403.04	2825.34	500.77
3	RhizoVital 42 (RV42)	Control	1701.24	12108.10	7349.37	2953.28	557.31
		RP	1593.45	9317.95	6553.81	2928.55	492.43
4	RhizoVitall 45	Control	1688.71	11730.16	8536.38	3395.25	593.36
		RP	1647.74	11374.37	6611.01	2845.27	583.20
5	MUCI	Control	1682.11	10629.77	7496.21	3102.95	490.31
		RP	1493.91	13141.45	5388.80	2495.47	461.46
6	RV42 + MUCI	Control	1446.92	9702.88	7896.33	2903.30	558.75
		RP	1248.07	13055.06	4245.53	1968.34	434.57
7	SuperFifty	Control	1650.76	11603.21	8204.55	3155.38	658.79
		RP	1561.19	11797.84	5886.88	2627.98	587.03
8	NemaTec	Control	1535.93	10649.94	7792.99	3137.75	613.51
		RP	1587.07	9589.94	5926.79	2857.55	518.96
9	LamVita	Control	1687.59	11489.67	7880.68	3051.21	676.53
		RP	1532.64	9895.92	5713.23	2588.97	573.26
10	Biological Fertilizer OD	Control	1606.45	11827.26	7298.17	2862.14	452.95
		RP	1641.96	9835.39	6522.27	2947.29	568.79
11	Trichoderma OMG	Control	1795.07	13304.43	7045.85	2860.79	498.57
		RP	1335.05	11132.86	4448.15	2254.23	415.58

*RP~ Rock Phosphate

Remark - Mean value for each parameter is used as basis comparison value.

Appendix- iii Measurement of element content in the aboveground dry biomass between Control and Rock-Phosphate

Sr. no	Treatment		Elements content in the aboveground dry biomass (mg/kg)					
			Fe	Cu	Zn	Mn	B	Ni
1	Control	Ctrl*	96.69	5.90	31.42	74.15	15.71	3.24
		RP**	112.48	5.07	21.35	62.09	13.74	3.39
2	Proradix	Ctrl	89.29	5.48	25.30	85.29	13.47	2.73
		RP	104.09	4.48	26.12	60.16	15.40	2.14
3	RhizoVital 42 (RV42)	Ctrl	68.52	5.73	26.63	79.88	14.71	2.01
		RP	70.33	4.80	26.29	62.09	15.06	1.79
4	RhizoVitall 45	Ctrl	62.65	5.79	31.87	78.93	15.97	0.54
		RP	71.95	4.85	26.92	64.01	15.48	1.64
5	MUCI	Ctrl	137.34	4.75	24.60	81.42	12.90	3.14
		RP	46.70	4.08	19.80	56.60	13.60	1.50
6	RV42 + MUCI	Ctrl	127.75	4.16	21.96	74.50	19.31	0.34
		RP	46.81	3.53	15.12	48.67	9.62	0.11
7	SuperFifty	Ctrl	68.19	5.25	26.81	77.38	14.84	0.49
		RP	53.52	4.90	22.87	62.77	14.98	1.90
8	NemaTec	Ctrl	670.76	5.15	27.10	77.54	14.26	0.59
		RP	51.02	5.15	23.40	68.22	12.64	2.08
9	LamVita	Ctrl	73.46	5.71	29.52	76.34	14.63	2.57
		RP	57.17	4.84	24.70	67.74	16.66	0.47
10	Biological Fertilizer OD	Ctrl	66.22	4.99	24.00	58.93	15.46	2.50
		RP	55.39	5.32	26.97	74.66	14.01	2.18
11	Trichoderma OMG	Ctrl	59.35	5.29	27.36	55.46	13.57	1.85
		RP	51.43	3.90	20.92	47.59	7.37	1.31

*Ctrl ~ Control
**RP ~ Rock Phosphate

Remark - Mean value for each parameter is used as basis comparison value.

Appendix- iv Measurement of element uptake by aboveground dry biomass between Control and Rock-Phosphate

Sr. no	Treatment		Element uptake by aboveground dry biomass				
			P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	S (µg/kg)
1	Control	Control	3.11	21.53	14.50	5.54	1008.95
		RP*	2.87	20.92	11.07	4.86	1036.25
2	Proradix	Control	2.93	22.00	12.51	4.89	936.28
		RP	3.08	18.03	12.65	5.61	996.20
3	RhizoVital 42 (RV42)	Control	2.74	19.70	11.81	4.74	898.30
		RP	3.33	19.54	13.66	6.10	1025.54
4	RhizoVitall 45	Control	2.63	18.21	13.31	5.29	922.65
		RP	3.15	21.90	12.62	5.43	1116.14
5	MUCI	Control	2.31	14.60	10.29	4.26	672.53
		RP	2.49	20.84	9.00	4.17	768.42
6	RV42 + MUCI	Control	2.05	13.68	11.21	4.13	787.57
		RP	2.24	23.50	7.65	3.54	780.14
7	SuperFifty	Control	2.53	17.73	12.58	4.83	1009.53
		RP	2.78	20.96	10.51	4.68	1044.82
8	NemaTec	Control	2.57	17.77	13.05	5.26	1021.76
		RP	3.47	21.00	12.90	6.22	1130.88
9	LamVita	Control	2.64	17.92	12.32	4.78	1057.57
		RP	3.28	21.51	12.32	5.57	1232.12
10	Biological Fertilizer OD	Control	2.82	20.87	12.79	4.99	788.28
		RP	3.16	19.20	12.72	5.73	1097.01
11	Trichoderma OMG	Control	3.03	22.28	11.88	4.84	835.19
		RP	2.62	22.64	8.78	4.44	814.99

*RP ~ Rock Phosphate

Remark - Mean value for each parameter is used as basis comparison value.

Appendix- v Measurement of element uptake by aboveground dry biomass between Control and Rock-Phosphate

Sr. no	Treatment		Elements uptake by aboveground dry biomass (µg/kg)					
			Fe	Cu	Zn	Mn	B	Ni
1	Control	Ctrl*	170.27	10.38	54.86	131.50	27.60	5.70
		RP**	207.25	9.33	38.96	114.04	25.09	6.29
2	Proradix	Ctrl	164.56	10.10	47.03	159.37	25.27	5.13
		RP	204.10	8.92	51.51	119.23	30.54	4.22
3	RhizoVital 42 (RV42)	Ctrl	110.37	9.22	43.10	129.40	23.79	3.26
		RP	147.56	10.03	54.92	129.60	31.25	3.76
4	RhizoVitall 45	Ctrl	97.50	9.00	49.77	122.95	24.94	0.83
		RP	143.52	9.28	51.08	123.16	29.81	3.12
5	MUCI	Ctrl	190.12	6.53	33.80	111.80	17.74	4.32
		RP	78.10	6.78	33.05	94.92	22.66	2.55
6	RV42 + MUCI	Ctrl	176.54	5.88	31.16	106.30	28.09	0.47
		RP	83.65	6.35	27.05	87.97	17.39	0.19
7	SuperFifty	Ctrl	104.66	8.05	40.96	118.76	22.75	0.72
		RP	95.03	8.73	41.08	112.06	26.63	3.39
8	NemaTec	Ctrl	101.00	8.54	45.40	129.35	24.04	0.99
		RP	111.42	11.22	50.86	148.40	27.70	4.58
9	LamVita	Ctrl	114.08	8.92	46.34	119.58	22.90	4.01
		RP	122.59	10.35	53.21	147.75	35.65	0.92
10	Biological Fertilizer OD	Ctrl	116.94	8.79	42.05	104.64	27.59	4.39
		RP	106.41	10.25	52.56	146.15	27.01	4.28
11	Trichoderma OMG	Ctrl	99.66	8.92	46.53	92.56	22.91	3.12
		RP	99.86	7.62	40.90	94.79	14.72	2.53

*Ctrl ~ Control
**RP ~ Rock Phosphate

Remark - Mean value for each parameter is used as basis comparison value.