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AKDENIZ UNIVERSITY

THE EFFECT OF *Bacillus sp.* MIXTURE ON BIOMASS PRODUCTION AND CHLOROPHYLL CONTENT OF LETTUCE (*Lactuca sativa*)

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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

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Bu tez …. /…../201…. tarihinde jüri tarafından Oybirliği / ~~Oyçokluğu~~ ile kabul edilmiştir.

Prof. Dr. Faik KANTAR (Supervisor)

Assoc. Prof. Dr. Şerife Evrim ARICI

Assoc. Prof. Dr. Hüseyin ÇANCI

ÖZET

Bacillus sp. BAKTERİ KARIŞIMININ MARUL'DA (*Lactuca Sativa*) BİYOMAS ÜRETİMİ VE KLOROFİL MİKTARI ÜZERİNE ETKİSİ

İBRAHİM BOZMAZ

Yüksek Lisans Tezi, Tarımsal Biyoteknoloji Anabilim Dalı

Danışman: Prof. Dr. Faik KANTAR

Haziran 2018; 46 sayfa

Bu çalışmada *Bacillus subtilis* VKPM B-10641 (DSM 24613) ve *Bacillus amyloliquefaciens* VKPM B-10642 (DSM 24614) ve B-10643 (DSM 24615) bakteri karışımı uygulamasının sera koşullarında verim ve verim bileşenleri üzerine etkisini araştırmak üzere yapılmıştır.

Deneme, Kontrol (T1), Standart Kimyasal Gübre (T2) ve Standart Kimyasal Gübre + Mikrobiyal Gübre uygulamaları olmak üzere tamamen tesadüfi bloklarda 4 tekerrürlü olarak 2015 yılı baharında Akdeniz Üniversitesi Ziraat Fakültesi deneme seralarında gerçekleştirilmiştir. Kök uzunluğu, kök boğazı çapı, kök yaş ve kuru ağırlığı, gövde çapı, baş yüksekliği, baş çapı, yaprak sayısı, yaprak yaş ağırlığı, yaprak kuru madde yüzdesi, suda çözünür kuru madde miktarı, klorofil içeriği, yaprak rengi ve pazarlanabilir baş verimi ölçülmüştür.

Yapılan uygulamaların yaprak rengi ve pazarlanabilir baş verimi üzerine etkisi istatistiksel olarak önemli bulunmuştur. Uygulamaların yaprak yeşil renginin bir ölçüsü olan b değerinde kontrol uygulamasına göre 37,71 ± 0,46 b* değerle % 4 azalmaya ve pazarlanabilir baş veriminde artışa sebeb olduğu bulunmuştur. Mikrobiyal gübre ve kimyasal gübrenin birlikte uygulandığı parsellerde pazarlanabilir baş verimi ortalama 2808,38 ± 154,80 kg/dekar ile kontrol uygulamasına göre 20 %, standart kimyasal gübre uygulamasına göre ise 11 % daha fazla bulunmuştur. Sadece standart kimyasal gübre uygulamasındaki ve mikrobiyal + kimyasal gübre uygulamasındaki verim artış oranlarına göre, marul pazarlanabilir baş verimini arttırmak için mikrobiyal ve kimyasal gübreler birlikte kullanılabilir.

ANAHTAR KELİMELER: *Bacilllus subtilis, Bacillus amyloliquefaciens,* Marul, Verim, Bitki gelişimi, Mikrobiyal gübre,

JÜRİ: Prof. Dr. Faik KANTAR

Doç. Dr. Şerife Evrim ARICI

Doç. Dr. Hüseyin ÇANCI

ABSTRACT

THE EFFECT OF *Bacillus sp.* MIXTURE ON BIOMASS PRODUCTION AND CHLOROPHYLL CONTENT OF LETTUCE (*Lactuca sativa*)

İBRAHİM BOZMAZ

MSc Thesis in Agricultural Biotechnology

Supervisor: Prof. Dr. Faik KANTAR

June 2018; 46 pages

This study was carried out in order to investigate the effects of *Bacillus subtilis* VKPM B-10641 (DSM 24613) and *Bacillus amyloliquefaciens* VKPM B-10642 (DSM 24614) and B-10643 (DSM 24615) mixture applications on the yield and yield components of lettuce (*Lactuca sativa* L. cv. Funly) under greenhouse conditions.

The experiment investigated three treatments as Control (T1), Standard Chemical Fertilizer Application (T2) and Standard Chemical Fertilizer Application + Microbial Fertilizer Application (T3) in a Completely Randomized Blocks with 4 replications on experimental farm of Akdeniz University, Faculty of Agriculture in Antalya in spring in 2015. Plant growth parameters of root length, root collar diameter, root fresh and dry weights, stem diameter, head height, head diameter, number of leaves, fresh weight of leaves, dry matter percentage of leaves, brix, chlorophyll content, leaf color and marketable head yield were measured.

The effect of treatments was found significant on leaf color and marketable head yield with a decrease on green color parameter b value of 37,71 ± 0,46 b* which was 4 % less than control treatment and an increase in total yield. As a combined effect of all traits on commercial yield, microbial and chemical fertilizer application gave the highest marketable head yield. Total yield of microbial and chemical fertilizer applied blocks were 2808,38 ± 154,80 kg/decare and 20 % higher than control and 11 % higher than chemical fertilizer applied treatment. According to the increase rates by chemical fertilizer application alone and microbial and chemical fertilizer combination, it is suggested that microbial fertilizers in combination with chemical fertilizers may be used in order to increase lettuce yields.

KEYWORDS: *Bacilllus subtilis*, *Bacillus amyloliquefaciens*, PGPR, Lettuce, Yield, Plant growth, Microbial fertilizer

COMMITTEE: Prof. Dr. Faik KANTAR

Assoc. Prof. Dr. Şerife Evrim ARICI

Assoc. Prof. Dr. Hüseyin ÇANCI

PREFACE

Continuous growth of human population is one of biggest challenges for us to face while we are producing foods to feed this population. When we think about other challenges like loss of arable lands and extreme environmental conditions coming with climate change, it is crucial that we have to keep innovating new technics and technologies and beside that we have to increase efficiency of what is was already innovated. This efficiency must be considered for any means of agriculture.

With this sense of being efficient, we can take agrochemicals as they are main source of nutrients and agents to fight against pest and diseases for agricultural production. It is well known that those chemical applications in agriculture has a lot of side effects to the nature especially when they are misused. Since it is not easily affordable to find an alternative for chemicals, being efficient on them becomes must. In this study it was targeted to find a way to increase yield by being effective on chemical use with the help of a microbial fertilizer mixture of *Bacillus subtilis* and *Bacillus amyloliquefaciens* strains.

I would like to express my gratitude to my supervisor Prof. Dr. Faik KANTAR for his support and contribution to this experiment, his supervision and candid feedbacks in every respect. I also want to thank to all my professors who I have attended their classes during my Master study.

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ACADEMIC DECLARATION

AKADEMİK BEYAN

I declare that " THE EFFECT OF Bacillus sp. MIXTURE ON BIOMASS PRODUCTION AND CHLOROPHIL CONTENT OF LETTUCE (*Lactuca sativa*)", which I have presented as my Master Thesis, is written in accordance with academic rules and ethical values and I declare that I show the source of all information which is not belong to me in this study.

Yüksek Lisans Tezi olarak sunduğum "*Bacillus sp.* BAKTERİ KARIŞIMININ MARUL'DA (*Lactuca Sativa*) BİYOMAS ÜRETİMİ VE KLOROFİL MİKTARI ÜZERİNE ETKİSİ" adlı bu çalışmanın, akademik kurallar ve etik değerlere uygun olarak yazıldığını belirtir, bu tez çalışmasında bana ait olmayan tüm bilgilerin kaynağını gösterdiğimi beyan ederim.

……/……../…….

İbrahim BOZMAZ

SYMBOLS AND ABBREVIATIONS

Symbols

pH : negative algorithm of hydrogen ion concentration
K : potassium
Mn : manganese
Mg : magnesium
Fe : iron
Ca : calcium
Zn : zinc
Cu : cupper
P : phosphor
N : nitrogen
% : percentage

Abbreviations

PGPR : plant growth promoting rhizobacteria
Ml : milliliter
L : liter
mg : milligram
mm : millimeter
ppm : parts per million
m^2 : square meter
Kg : kilogram
Da : decares
Ha : hectare
g : gram
m : meter
cm : centimeter
Ec : electrical conductivity

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1. INTRODUCTION

Turkey is a country having big portion of its population working on agriculture to produce wide range of commercially valuable products in different regions having different climatic conditions. Vegetables have an important part in this production. Turkey has total production of 24.401.231 tons of vegetables in year 2016 and 478.442 tons of production comes from lettuce (FAOSTAT 2016).

Lettuce (*Lactuca sativa L.*) is a member of Asteraceae (*Compositeae*) family. It can be grown whole year around in open fields and under protections. There are different ideas among botanists and researchers about how it was spread to the world. Lettuce was cultivated for the first time in Egypt in 4500 B.C. and wild forms were spread on middle to south of Europe, from Canary Islands, Algeria, Ethiopia to Mesopotamia, west Asia, Caucasia, Kashmir and Nepal in north of India. It is accepted that lettuce has been produced at least for the last 2500 years in a wide range of areas including Europe, Asia and North Africa (Vural et al. 2000).

Lettuce is a vegetable which needs humid and cool weather for its growing condition and it can partly tolerate cold weathers. For this type of cool climate vegetables, monthly average temperature of 15-18°C are best temperatures for production. However, plant vegetation and growth can continue at maximum of 27-30°C and minimum of 2-4°C temperatures (Thompson 1957). 15°C of temperature is recommended for optimum germination of lettuce seeds. Since lettuce has short vegetation period, it can be grown all around Turkey. Varieties suitable for warmer conditions can be grown on highlands with an altitude of 1000-1500 m on summer times (Günay 2005).

Stem of lettuce starts just after soil surface with a shape like rosette with a bunch of leaves lowering in density as the stem goes up. It has a strong root system which can go quite deep into the soil. Production cycle is kept short to prevent full growth of the stem. The leaves of lettuce which is consumed as a vegetable, can have different shape and characteristic for different variety. Leaf shape of lettuce varies for its curly structure and grouped by smooth to little curly, medium curly and curly (Eşiyok 1996). Average number of 40-45 leaves form a structure called head of lettuce. Lettuce is commonly described as a long crop and depending on the variety after some certain days it starts to give flowers by the weather gets warmer. The flowers are hermaphrodite and pollination of those flowers are largely self-pollinated. (Anonymous 1996).

Constantly increasing world population will be facing shortage of foods, due to loss of arable lands by erosion, aridity or miss use for tourism and residential areas (Sevgican 2003; Yilmaz 2005). On the other hand, new tools and practices were developed in parallel to increasing world population, to increase yield and quality of crops (Aksoy 1999).

In addition, increasing surface of agricultural areas and increasing total yield per unit area by intensive use of chemical fertilizers are set as target (Dursun et al. 2010). This intensive and indiscriminate use of chemical fertilizers and pesticides for production and pest control, cause to ruin healthy structure of soil, increase on populations of pathogens and pests and environmental pollution. With an intensive use of agricultural chemicals, sustainable agriculture cannot be maintained, and many hazardous and toxic

chemicals are accumulated in agricultural ecosystems. These harmful chemicals put human health in danger by getting into soil, underground waters, plant structures and our foods (Saber 2001; Çakmakçı 2005).

The soil fertility is decreased by reckless and careless use of chemicals and loss of soil organic matter. These agricultural applications harming soil structure for higher yield, causing consumer pressure on growers for the loss of quality on final product, environmental pollution and threaten human health. Especially these types of products are not demanded on export market. That's why, an agricultural system without use of chemicals to produce healthy and clean food, is a must. In this aspect, sustainable agriculture becomes an important aspect all around the world. Sustainable and good agricultural practices are targeting the efficient and effective use of soil, water and botanical resources, protection of environment, food safety for community health care and hand down a good nature for the next generations. Benefits of biological applications become priority instead of chemicals with the new approach of sustainable agriculture (Merdin 2009).

Reasons for yield and quality loss are divided into two main factors of biotic and abiotic factors. Abiotic factors are for unfavorable environment and soil conditions. Biotic factors are for pathogens (like fungus, bacteria, virus, mycoplasma, etc.), pests and weeds. It is very important to increase the efficiency obtained from the unit area by carrying out the applications required to control these factors. However, chemical inputs are used intensively in plant production especially in the greenhouses. The most important reasons for this are; production of the same or relative species with high economic value, a suitable environment for disease agents and harmful factors in greenhouses and cultivation of varieties with high nutrient requirements (Tüzel and Gül 2008).

In recent years, opportunities to utilize biological applications instead of chemical use have gained importance in ensuring sustainability in agriculture. Beneficial microorganisms have begun to be exploited to increase the resistance of plants to biotic and abiotic stress conditions and to improve plant growth and yield (Armstrong 2001; Postma et al. 2001; Deniel et al. 2006; Gül et al. 2007; 2008a; Gül et al. 2008b; 2008c; Kıdoğlu et al. 2007, 2008). These bacteria are known as Plant Growth Promoting Rhizobacteria (PGPR) and promotes vegetative and generative growth activity and promotes natural endurance of plants against many bacteria, fungus and viruses in various proportions of plants like vegetables, ornamental plants, some trees, cereals, etc. (Backman et al. 1997; Weller 1988; Wei et al. 1996).

PGPRs generally regulate plant growth by colonizing in the root system and suppress harmful rhizosphere microorganisms. PGPRs also provide very important contributions to seed germination, root development and plant utilization. These rhizobacteria can indirectly affect plant growth by producing growth hormones and modifying the microbial balance in the rhizosphere in favor of beneficial microorganisms, or by regulating the mineral content ratio. It suppresses bacterial, fungal and nematode diseases to a large extent and protects against viral diseases too (Sıddıqui 2006).

In recent years PGPR bacterial strains have begun to be used in different plants. In the studies carried out with *Bacillus* strains on wheat (De Freitas 2000), maize (Pal 1998), barley (Çakmakçı et al. 1999), sugar beet (Şahin et al. 2004) and spinach

(Çakmakçı et al. 2007b) showed an important increase on yield. PGPR inoculation has supported the growth of strawberry and peanut (Kokalis-Burelle 2003), summer wheat (Walley and Germida 1997), spinach (Çakmakçı et al. 2007b), lettuce (Barazani and Friedman 1999; Arkhipova et al. 2005). Nitrogen-fixating and phosphate-dissolving bacterial applications increase available natural population of bacteria and the amount and uptake of N and P in the rhizosphere (Canbolat et al. 2006; Çakmakçı et al. 2007a).

PGPR activity has been shown variation according to plant and bacteria types inoculated, measured plant parameters, growing conditions, soil characteristics and especially soil organic matter content (Çakmakçı et al. 2006). Mixed inoculation of bacteria increases bacterial activity and provides a more balanced intake of nutrients (Şahin et al. 2004). Nitrogen-fixation and phosphate-dissolving bacteria inoculation has been shown to be an alternative to mineral fertilization in terms of yield increase, cost and pollution reduction especially in greenhouse conditions where water and temperature are more favorable for bacteria (Çakmakçı 2002). Bacteria have been shown to encourage plant growth especially early stages of vegetation (Şahin et al. 2004), suggesting that biological fertilizers can give more favorable results to plants which are grown for their leaves. Quality of sugar beet was found negatively affected by only use of mineral nitrogen fertilizer but, it was more balanced with bacterial inoculation (Çakmakçı 2002). According to these research findings, it can be expected that if appropriate strains are identified, the growth of cultured plants can be positively influenced by providing more balanced intake of the other elements.

In this study, *Bacillus subtilis VKPM B-10641 (DSM 246]3), Bacillus amyloliquefaciens VWM 8-10642 (DSM 24614) and 8-]0643 (DSM 24615)* were used as microbial fertilizers with antibacterial properties which, result from the production of 70 different natural antibiotics. In addition, these bacteria create wide range of enzymes: amyloplastic, cellulose lytic, proteoclastic enzymes which break down the organic materials of the soil and make the soil fertile. The microbial fertilizer that we use is highly effective against various infections caused by bacteria and fungi such as *Didymella applanata, Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Fusarium graminearum, Fusarium moniliforme, Fusarium asporotrichiella, Alternaria alternata, Rhizoctonia solani, Phytophthora infestans, Bipolaria ribis*, *Pseudomonas* and *Erwinia.*

2. THEORY AND LITERATURE STUDY

The rhizosphere of plants is a region with intense microbial activity (Altın and Tayyar 2005; Bolwerk 2005). It is known that carbon sources such as organic acids, sugars and amino acids secreted by plant roots promote microorganism activity in rhizosphere (Bolwerk 2005).

There are interactions between the rhizosphere microorganisms themselves and between microorganisms and roots, and these interactions may be useful, ineffective or harmful (Lynch and Whipps 1991). The beneficial interactions between microorganisms and plant roots can be grouped into 4 groups; (1) increase the amount of nutrients available for plants, (2) increase plant development by producing auxin, (3) biological cleansing of rhizosphere, and (4) reduce plant disease outbreaks (Bolwerk 2005).

Rhizosphere is the soil environment where the plant root is available and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro- and micronutrients are extracted. The microbial population present in the rhizosphere is relatively different from that of its surroundings due to the presence of root exudates that function as a source of nutrients for microbial growth (Burdman et al. 2000). Weller and Thomashow (2007) prove that the narrow rhizosphere zone is rich in nutrients for microbes compared to the bulk soil; this is shown by the quantity of bacteria that are present surrounding the roots of the plants, generally 10 to 100 times higher than in the bulk soil.

The microbial colonization of rhizosphere includes bacteria, fungi, actinomycetes, protozoa, and algae. However, bacteria are the most abundant microbial present in the rhizosphere (Kaymak 2010). The enhancement of plant growth by the application of these microbial populations is well known and proven (Saharan and Nehra 2011; Bhattacharyya and Jha 2012). Kloepper and Schroth (1978) introduced the term “plant growth promoting rhizobacteria (PGPR)” for these beneficial microbes, by which paving the way for greater discoveries on PGPR. PGPR are not only associated with the root to exert beneficial effects on plant development but also have positive effects on controlling phytopathogenic microorganisms (Kloepper et al. 1980; Son et al. 2014).

These bacteria are known as Plant Growth Promoting Rhizobacteria (PGPR), which has been shown to increase vegetative and generative development in plants at varying rates and to provide protection against many bacterial, fungal and viral plant diseases by stimulating natural defense mechanism of plants (Backman et al. 1997; Weller 1988; Wei et al. 1996).

PGPR can be separated into symbiotic bacteria, which can live inside the plants and exchange metabolites with plants, or they live outside the plant cells, based on their interaction with plants (Gray and Smith 2005). The working mechanisms of PGPR can also be divided directly and indirectly. Biofertilization, stimulation of root growth, rhizoremediation, and plant stress control are the direct mechanisms of PGPRs. Rhizobacteria can affect biological control mechanism of plants indirectly as plant growth promoter by reducing the impact of diseases, which include antibiosis, induction of systemic resistance, and competition for nutrients and niches (Egamberdieva and Lugtenberg 2014).

According to Tilak et al. (2005), many of the PGPR group bacteria were also very good as biological control agents. These bacteria can achieve a considerable success to fight against plant diseases and especially soil-borne diseases. Within this concept, there are many examples in the world within as biopesticide. PGPRs are considered indispensable items of agricultural techniques such as Organic Agriculture and Integrated Product Management, as bio fertilizer and biopesticide in biological control in terms of yield increasing properties.

By the middle of the 20th century, the Soviet Union and India were working on the effects of PGPR on different products. Although the results obtained from different field trials made were inconsistent, it was reported that yield increases of 50-70% compared to the control were achieved. Although the mechanism of PGPR in promoting plant growth during this period is not well known, these trials have provided clues to the appropriate conditions for bacterial colonization and plant growth in target plants. It has been determined that PGPR was beneficial to plant growth by germination rate, root growth, yield, leaf area, chlorophyll content, Mg, N content, protein, hydraulic activity, stiffness, shoot and root weights and delayed formation of the fissure layer (Lucy et al. 2004).

According to a study on banana it was found that PGPRs increased number of roots, root length and weight and nitrogen concentration of roots. Another increase was also found on chlorophyll content which measured by SPAD 502, MINOLTATM Camera Ltd Japan, and on the weight of leaves (Baset Mia et al. 2010).

Main source of agricultural yield reduction is considered to be abiotic stresses, which varies for the intensity depending on the type of soil (deficiency of hormonal and nutritional imbalances) and plant factors (physiological disorders such as susceptibility to diseases, etc.) (Nadeem et al. 2010). Nautiyal et al. (2008) demonstrated the increase in the antioxidant capacity and growth by the *Bacillus lentimorbus* strain on the edible parts of spinach, carrots, and lettuce.

Another major effect of PGPR on plants under abiotic stress conditions is the improvement of leaf water status, especially under salinity and drought stress (Ahmad et al. 2013, Naveed et al. 2014). Sarma and Saikia (2014) reported that *Pseudomonas aeruginosa* strain has improved the growth of *Vigna radiata* (mung beans) plants under drought conditions. The ability of plants in utilizing water for growth depends on their stomatal apertures. The stomatal on the plant leaf functions to balance the water content in leaf and water uptake by the roots. Ahmad et al. (2013) and Naveed et al. (2014) reported that the stomatal conductance (water vapor exiting through the stomata leaf) of plant leaf was higher in PGPR inoculated plants than non-PGPR inoculated ones under drought conditions. The finding from both studies proves that PGPR-inoculated plants tend to improve the water-use efficiency of plants. This finding could be beneficial to the environment in terms of reducing excessive usage of water.

The main mechanism of increasing resistance to abiotic and biotic stress elements is achieved by promoting plant growth. Phosphorus compounds found in the soil and applied to the soil are undergoing a fixation in the form of Ca compounds (Yadaw and Dadarwal 1997; Çakmakçı et al. 2008; Karaçal and Tüfenkçi 2010). PGPRs increase inorganic and organic phosphorus solubility with microbial metabolites, which promote

plant development. In addition, it increases the uptake of nutrients by producing organic acid and acid phosphatase (Kucey et al. 1989; Kumar and Narula 1999; Puente et al. 2004; Çakmakçı et al. 2005). It was also emphasized that PGPR applications have a positive effect on the intake of plant nutrients, yield and yield components in many studies (Kucey et al. 1989; Kumar and Narula 1999; Puente et al. 2004; Çakmakçı et al. 2005; Gül et al. 2007; Dursun et al. 2008; Seymen et al. 2010).

Root bacteria stimulating plant growth, synthesize siderophores, which are water-soluble molecules with high cohesion and low molecular weight Fe + 3 ions to obtain iron in limited quantities in the environment (Altın and Tayyar 2005). Siderophore means iron carrier, which take surrounding iron ions and increases the iron uptake of the plant and prevents pathogens from developing by binding the iron in the environment. With this biocontrol mechanism by inhibiting pathogens, plant development is affected positively (Özaktan and Bora 1994; Erdal 2005). Rhizobacteria increase plant resistance to pathogens and plant growth by using several mechanisms, such as competition for food and living space, the production of pathogen-inhibiting chemicals, the production of siderophore, and the promotion of plant resistance against pathogens (Compant et al. 2005).

Available nutrient concentration of rhizosphere can be increased by the nutrient fixing role of PGPRs, thus prevent nutrients to leach out (Choudhary et al. 2011). As an example, nitrogen is one of the most limiting nutrient for plants, which is needed for amino acid and protein synthesis. Atmospheric nitrogen which prokaryotes can turn into organic form for plants to be able to assimilate (Lloret et al. 2005; Raymond et al. 2004). A rare example of a free-living nitrogen-fixing organism is *Azospirillum*, often associated with cereals in temperate zones and also reported to be able to improve rice crop yields (Tejera et al. 2005).

Zapata et al. (2003) reported that, nine types of lettuce seeds were germinated under control and saline (150 mM NaCl) conditions. At the end of the experiment, salt stress effects on germination, growth, ethylene production, respiration rate and polyamine levels were investigated. Studies have shown that germination was reduced, and growth was delayed in all strains studied under salt stress. Respiration speed and ethylene production increased in nine types.

Mayak et al. (2004), indicated that Rhizobacteria increased resistance to salt stress in tomatoes. They took soil samples from the Arava region of southern Israel and applied seven strains of plant growth promoting bacteria to increase plant growth at 43 mM NaCl for 7 weeks. *Achromobacter piechaudii* was selected for further study as the most effective strain. In the presence of 172 mM NaCl salt, this bacterium increased the fresh and dry weights of the tomato seedlings and the water use efficiency of the seedlings. The result was that efficient farming systems can be developed for saline environments.

Kıdoğlu et al. (2007) reported that root bacteria stimulating plant growth could be used to control fungal, bacterial and viral pathogens, as well as biological agents against root nematodes, increased resistance to biotic and abiotic stress conditions, and positive effects on plant growth and yield. In this study, which was conducted to benefit from these positive contributions of root bacterium stimulating plant growth, the effect of root bacterium on seed germination *in vitro* and seedling growth *in vivo* was determined. From

the tested local isolates, 18/1 K, 66/3 and 70 significantly increased seedling growth.

At high concentration, IAA promotes ethylene production by stimulating an important step in ethylene synthesis which formation of ACC (1-amino-cyclopropone-1-carboxylic acid) (Wang et al. 2000). At lower levels of ethylene, root formation is increased, and root extension is induced. The high level of ethylene produced by rapidly growing roots prevents root extension (Pal et al. 2000).

Synthesis of plant hormones such as cytokinin, IAA and gibberellin are one of the mechanisms of plant growth enhancement of root bacteria (Loper and Schroth 1986; Tang 1994; Salamone and Wodzinski 1997). Among plant hormones, IAA (indole-3-acetic acid) and ethylene predominate. As is known, IAA encourages cell expansion and prolongation in plants. Increased length and development of root by IAA synthesized by bacteria, facilitates nutrient uptake from the soil by the larger root surface area of the plant (Vessey 2003).

In a study for the effects of root bacteria on the development of lettuce seedlings, 6 different local root bacterium isolates (18 / 1K: *Pseudomonas putida*, 21 / 1K: *Enterobacter cloacae*, 62: *Serratia marcescens*, 70: *Pseudomonas fluorescens*, 66/3: *Bacillus spp.* 180: *Pseudomonas putida*), 2 different exported commercial isolates (*Bacillus amyloliquefaciens* FZB24, *Bacillus amyloliquefaciens* FZB42) and control application were compared and root bacterium was found to be effective in increasing head and root growth of the lettuce seedlings. 66/3 (*Bacillus spp*), 70 (*Pseudomonas fluorescens*) and 18 / 1K (*Pseudomonas* 8 *putida*) from the tested local isolates were selected for further study because of the significant increase in seedling development (Kıdoğlu et al. 2007).

Jeon et al. (2003) used a poor soil on the coast of a lake in Korea in their study. *P. flourescens* and *B. megaterium* strains had contributed significantly to plant growth, suggesting that phytohormones and particularly indole acetic acid produced by these strains may be related to the dissolution of phosphates which was insoluble in soil. Barazani and Friedman (1999) reported in a study they made in Israel that benefits of PGPR strains or deleterious rizobacteria (DRB) effects are dependent on the amount of auxin they produce. In the study carried out on lettuce plants, a large amount of indole acetic acid producing *Micrococcus luteus, Streptoverticillium sp*., *Gluconobacter sp.* and *P. putida* bacteria inoculation suppressed root development and inoculation of such bacteria like *Agrobacterium sp*., *Alcaligenes piechaudii*, *Comamonas acidovorans* were triggered root development by producing lower levels of indole acetic acid than others. Researchers have suggested that PGPR strains produce growth-promoting secretions other than indoleacetic acid (Barazani and Friedman 1999).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Microbial fertilizer

A bacterial mixture with concentration of *Bacillus subtilis* VKPM B-10641 (DSM 24613) and *Bacillus amyloliquefaciens* VKPM B-10642 (DSM 24614) and B-10643 (DSM 24615) in equal ratios, which were isolated from the soil of the Siberian environmentally pristine areas and selected by the developer. Mixture is a modern, biologically multifunctional preparation with a complex effect on cultivated plants, soil and detrimental organisms which was obtained from a biological fertilizer company named "Altay Bio Gübre Sanayi ve Ticaret Limited Şirketi" and applied as microbial fertilizer.

3.1.2. Plant material

A commercial green leaf lettuce variety Funly (from Syngenta) was used as plant material in present study. The lettuce cultivar Funly is suitable for growing in every season in greenhouse or open field and has light green leaves with high tolerance against tip burn and bolting.

3.1.3. Research field

This research was held in the greenhouse of Agricultural Faculty of Akdeniz University in March-April 2015.

3.1.4. Greenhouse soil structure

The greenhouse soil samples were tested for traits given down below (Table 3.1.4.1).

Soil texture: Hydrometer technique was used to determine the level of clay, silt and sand compound of soil.

Soil reaction (pH): 20 gr soil sample and 40 ml distilled water were mixed to make a solution with 1:2 ratio. The solution was mixed time to time with a glass rod and kept 30 minutes. After that, Neel pH meter with glass electrodes was used to determine pH.

Electrical conductivity (EC): The same solution prepared for pH test was used for EC measurement.

Calcium carbonate (%): 0,5 gr of soil sample was treated with hydrochloric acid (10%) and total calcium level was calculated according to the measured volume of carbon dioxide by Scheibler Calcimeter.

Exchangeable cations (Ca, Na, K): Cations were calculated with extraction solution by a flammenphotometer.

Total N: Kjeldal method was used to determine total nitrogen compound.

Nitrate determination: Method developed by MULVANEY (1996) was used to determine nitrate.

Phosphorus analysis (P): Sodium bicarbonate method was used to determine soil phosphorus compound.

Soil temperature: An analog thermometer was placed 15 cm under soil surface and temperatures were recorded every day.

Table 3.1.4.1. Analysis of experimental soil

Trait	**Value**	
Texture	Clay loam	
Ph (1:2,5)	7,62	Slightly alkali
EC (1:2,5) µS/cm	110	Very low
Lime (%)	17,7	High
Organic Matter (%)	2,1	Optimum
Total N (%)	0,09	Optimum
P (%)	0,0013	Low
K (%)	0,19	Very high
Ca (%)	0,4	High
Mg (%)	0,09	Optimum
Mn (mg/kg)	2,67	Sufficient
Zn (mg/kg)	0,47	Low
Cu (mg/kg)	0,25	Sufficient
Fe (mg/kg)	1,2	Low

Table 3.1.4.2. Analysis of water used in experiment irrigation

EC (dS/m)	**pH**	**K (ppm)**	**Ca (ppm)**	**Mg (ppm)**	**Na (ppm)**
0,699	7,12	2,3	87	17	21

*Water analysis were done by department of Agricultural Construction and Irrigation at Faculty of Agriculture in Akdeniz University

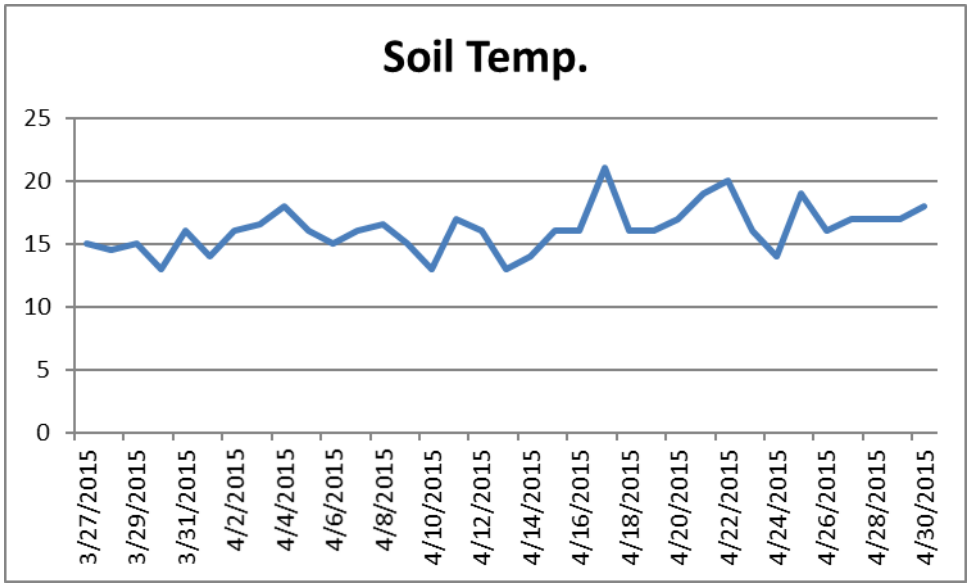

Figure 3.1.4. Soil temperature at the experimental site during vegetation period.

3.2. Methods

3.2.1. Treatments

In the experiment three treatments were investigated as Control with no application (T1), Standard Chemical Fertilizer treatment of NPK for 2 times after transplantation (T2), Microbial Fertilizer of *Bacilllus subtilis and Bacillus amyloliquefaciens* Mixture + Standard Chemical Fertilizer application of NPK (T3).

Bacteria mixture was applied to seedlings in trays, just before transplantation. NPK fertilizers were given in the same amount and at the same time to T2 and T3 applications.

3.2.2. Field plan

Field plan was generated according to randomized block design method with 4 replications. Each block had 1,2 m width and 10 m length with 3 rows of plantation. Plantation distance in-between rows and on the rows was 40 cm. Total 12 Blocks were transplanted on three lines with distance of 0,8 m on the line and 0,5 m in-between lines in total are of 195,04 m2 (4,6m x 42,4m).

3.2.3. Application details

The field was prepared to get uniform texture after plowing and drip irrigation system was set. To eliminate unwanted contamination, all replications of T1 and T2 blocks were transplanted first. Bacteria mixture solution was prepared with 1 ml of mixture for 10 L of water. One hour before transplantation, 15-20 ml of solution was applied to all seedlings of T3 blocks. In the growing period of plants, regular controls and irrigation was done together with needed agricultural applications (cleaning weed, spraying pesticides). NPK fertilizers were prepared by using 15(N)-5(P)-30(K) kg for 0,1

ha ratio. The fertilizers were divided into two and given at two times to all T2 and T3 blocks (Figure 3.2.3.).

Figure 3.2.3. Experiment blocks

3.3. Observation and data collection

During and after vegetation period, all morphological observations listed below and weighing of total yield were done.

3.3.1. Root length (cm)

5 plants from each plot were carefully pulled out from soil and cut from root collar. These cleaned roots were measured in length by a ruler.

3.3.2. Root collar diameter (mm)

The same 5 plants from each plot were measured by a digital caliper (Figure 3.3.2.).

Figure 3.3.2. Digital caliper

3.3.3. Root fresh weight (g/plant)

5 plants from each plot were carefully pulled out from soil and cut from root collar. These cleaned roots were weight by a balance with sensitivity of ±0,1 g.

3.3.4. Root dry weight (g)

The same 5 roots after measuring fresh weight were kept in a stove at 65°C until they dry. These dried roots were weighted by a balance with sensitivity of ±0,1 g.

3.3.5. Stem diameter (mm)

Stems of 5 plants from each plot were measured by a digital caliper. Mean value was calculated and recorded in "mm".

3.3.6. Head height (cm)

Heads of 5 plants from each plot were measured from their root collars to tip of their heads by a ruler. Mean value was calculated and recorded in "cm".

3.3.7. Plant diameter (cm)

Heads of 5 plants from each plot were measured by an elastic measuring tape. Middle of the heads was selected to measure where the heads have the largest diameter.

3.3.8. Number of leaves

5 plants from each plot were harvested and number of leaves was counted one by one. Mean value was calculated and recorded in "number".

3.3.9. Fresh weight of leaves (g)

5 heads from each plot were harvested, cleaned from not marketable leaves and weighted by a balance with sensitivity of ±1 g. Mean values were calculated and recorded in "g".

3.3.10. Dry matter percentage of leaves (%)

Leaves harvested from 5 plants in each plot were weighted for their fresh weight and kept in a stove at 65°C until they dry. After drying leaves, dry weights were measured again. Mean value of dry weight was calculated for 100 g of fresh leave and recorded in "%".

3.3.11. Brix (%)

Dry matter was measured by a refractometer (Figure 3.3.11.) on 3 plants from each plot. Mean value was calculated and recorded in "%".

Figure 3.3.11. Atago Pal-1 digital refractometer

3.3.12. Chlorophyll content (SPAD)

Chlorophyll compound of leaves were measured in the field on 5 plants from each plot by handheld SPAD meter device of Konica-Minolta SPAD-502 (Figure 3.3.12.).

Figure 3.3.12. Konica-Minolta SPAD-502

3.3.13. Leaf color

Just before harvest, colors of leaves were measured in the field on 5 plants from each plot by handheld device of Minolta Chroma Meter.

3.3.14. Marketable head yield (kg/decare)

All heads in each plot were harvested, cleaned from not marketable leaves and weighted by a balance with sensitivity of ±1 g. Total mean yield for 0,1 ha was calculated by multiplying yield data from each plot and recorded in “kg”.

4. RESULTS AND DISCUSSION

4.1. Root Length

Treatment effect was not significant, but block effect was significant (P<0.018) on root length (Table 4.1.1). Interaction effect was also insignificant.

The average root length for control T1 was the highest with 15,45 cm among other treatments. Fertilizer treatment T2 was the second with 14,76 cm and third treatment of microbial and chemical fertilizer T3 was found the shortest with 14.65 cm on average root length (Table 4.1.2).

Table 4.1.1. Analysis of variance for the effect of microbial fertilizer on average root length (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	7,521	2	3,761	1,227	,302
Block	34,032	3	11,344	3,700	,018
Treatment * Block	27,084	6	4,514	1,472	,208
Error	147,172	48	3,066		
Total	13631,940	60			

Table 4.1.2. The effect of microbial fertilizer on average root length (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blocks				
	A	**B**	**C**	**D**	**Average (cm)**
T1- Control	14,7	17,1	13,8	16,2	15,5
T2- Chemical Fertilizer	14,3	15,9	15,4	13,4	14,8
T3- Microbial + Chemical Fertilizer	14,7	15,7	13,9	14,3	14,7
Average	14,6 a	16,2 b	14,4 a	14,6 a	

*Standard Error for Blocks = 0,6394

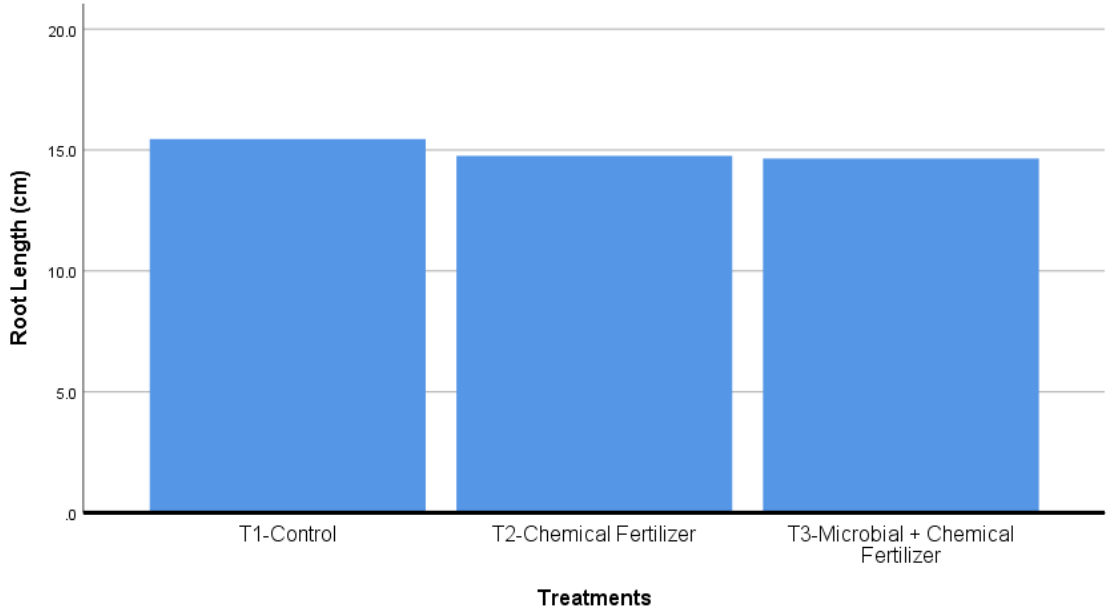

Figure 4.1. The effect of microbial fertilizer on average root length (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

4.2. Root Collar Diameter

The effect of treatment and blocks were not significant on root collar diameter, but interaction of them was significant (P<0.029) (Table 4.2.1.).

The average root collar diameter for control T1 was the highest with 22.63 mm among other treatments. Fertilizer treatment T2 was the second with 21.66 mm and third treatment of microbial and chemical fertilizer T3 was found the lowest with 20.97 mm on average root collar diameter (Table 4.2.2.).

Table 4.2.1. Analysis of variance for the effect of microbial fertilizer on average root collar diameter (mm) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	27,991	2	13,996	2,859	,067
Block	28,482	3	9,494	1,939	,136
Treatment * Block	76,647	6	12,775	2,610	,029
Error	234,978	48	4,895		
Total	28758,809	60			

Table 4.2.2. The effect of microbial fertilizer on average root collar diameter (mm) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	**Blok**				
	A	**B**	**C**	**D**	**Average (mm)**
T1- Control	21,36	21,71	23,00	24,46	22,63 b
T2- Chemical Fertilizer	20,87	21,16	23,47	21,13	21,66 ab
T3- Microbial + Chemical Fertilizer	22,27	19,85	18,91	22,84	20,97 a
Average	21,50 ab	20,91 a	21,79 ab	22,81 b	

*Standard Error for Treatments = 0,6997, **Standard Error for Blocks = 0,8079

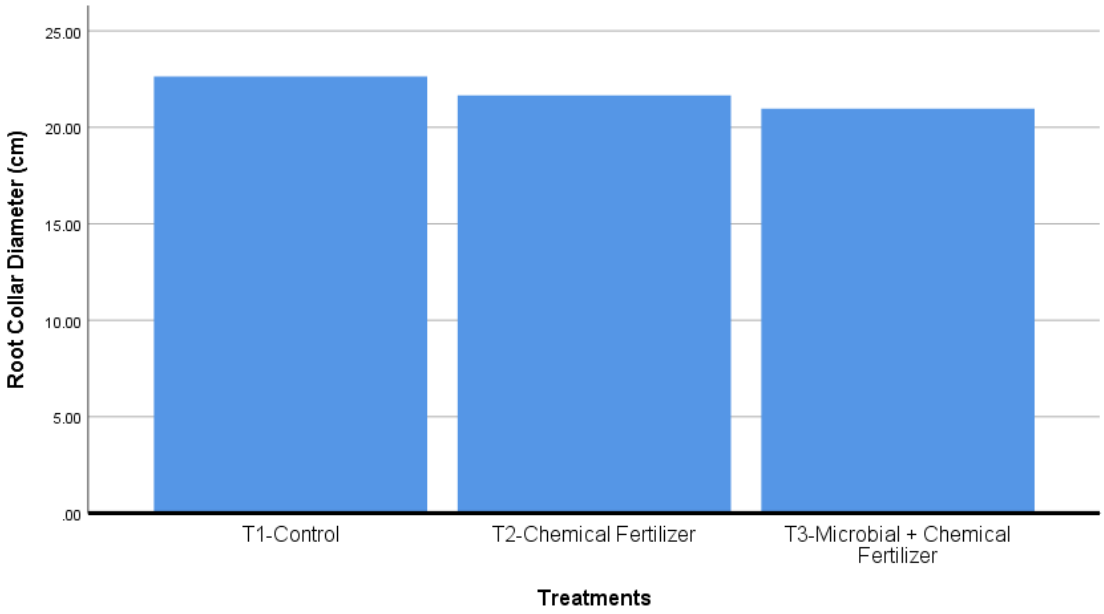

Figure 4.2. The effect of microbial fertilizer on average root collar diameter (mm) of green leaf lettuce variety (Funly) under greenhouse conditions

4.3. Root Fresh Weight

Treatment effect was not significant, but block effect was significant (P<0.001) on root fresh weight (Table 4.3.1.). Interaction effect was significant (P<0.000).

The average root fresh weight for control T1 was the highest with 21.33 g/plant among other treatments. Microbial and chemical fertilizer treatment T3 was the second with 20.77 g/plant and third treatment of Fertilizer T2 was found the lowest with 19.58 g/plant on average root fresh weight (Table 4.3.2.).

Table 4.3.1. Analysis of variance for the effect of microbial fertilizer on average root fresh weight (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	31,895	2	15,947	1,448	,245
Block	231,880	3	77,293	7,020	,001
Treatment * Block	687,553	6	114,592	10,408	,000
Error	528,486	48	11,010		
Total	26834,407	60			

Table 4.3.2. The effect of microbial fertilizer on average root fresh weight (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	**B**	**C**	**D**	**Average (g/plant)**
T1- Control	21,90	28,22	15,42	19,76	21,33
T2- Chemical Fertilizer	19,30	17,26	24,64	17,10	19,58
T3- Microbial + Chemical Fertilizer	25,22	23,05	14,78	20,03	20,77
Average	22,14 b	22,84 b	18,28 a	18,96 a	

*Standard Error for Treatments = 1,0493, **Standard Error for Blocks = 1,2116

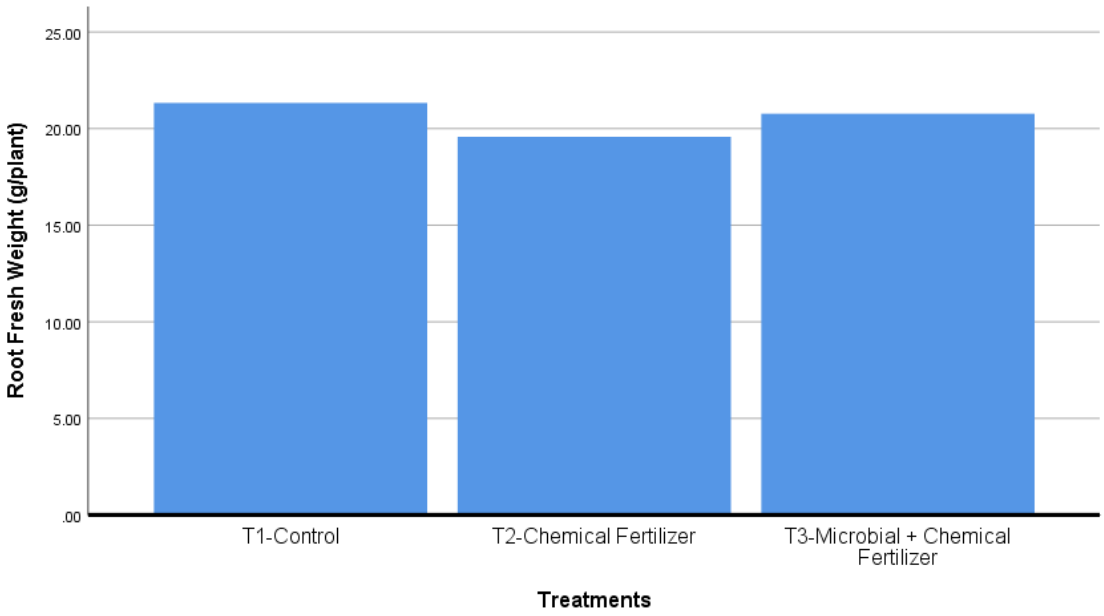

Figure 4.3. The effect of microbial fertilizer on average root fresh weight (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

4.4. Root Dry Weight

Treatment and block effect were not significant, but treatment*block interaction was significant (P<0.000) on root dry weight (Table 4.4.1).

The average root dry weight for control T1 was the highest with 7.35 g/plant among other treatments. Fertilizer treatment T2 was the second with 7.32 g/plant and third treatment of microbial and chemical fertilizer T3 was found the lowest with 7.29 g/plant on average root dry weight (Table 4.4.2.).

Table 4.4.1. Analysis of variance for the effect of microbial fertilizer on average root dry weight (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	,028	2	,014	,109	,897
Block	,762	3	,254	1,959	,133
Treatment * Block	4,198	6	,700	5,396	,000
Error	6,224	48	,130		
Total	3225,863	60			

Table 4.4.2. The effect of microbial fertilizer on average root dry weight (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	**Blok**				
	A	**B**	**C**	**D**	**Average (g/plant)**
T1- Control	7,44	7,72	6,99	7,23	7,35
T2- Chemical Fertilizer	7,13	7,30	7,83	7,03	7,32
T3- Microbial + Chemical Fertilizer	7,60	7,38	6,83	7,36	7,29
Average	7,39	7,47	7,21	7,21	

*Standard Error for Treatments = 0,1140, **Standard Error for Blocks = 0,1310

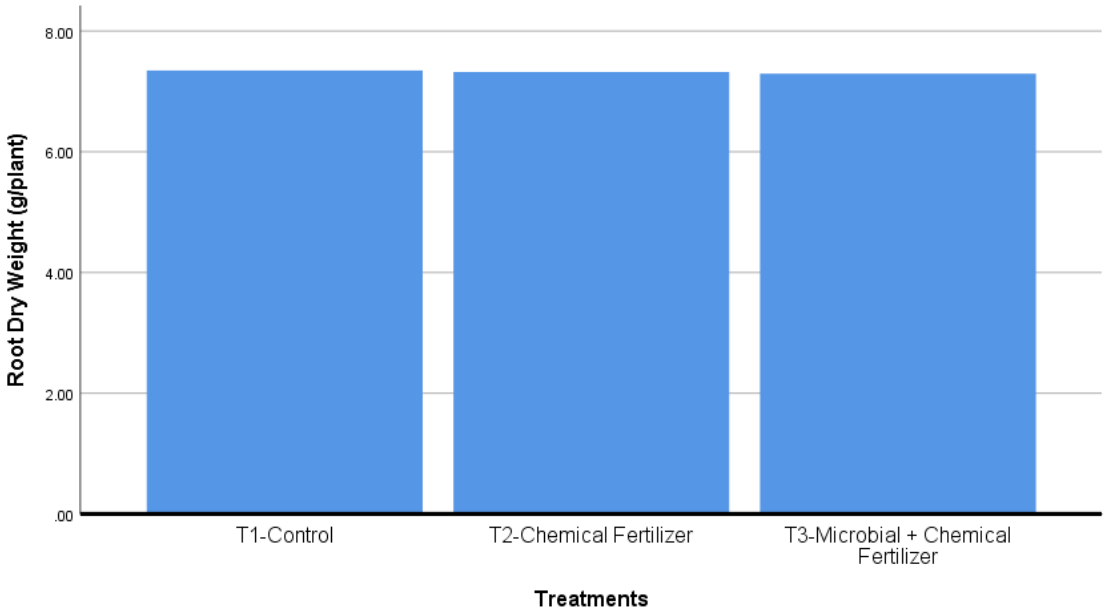

Figure 4.4. The effect of microbial fertilizer on average root dry weight (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

4.5. Stem Diameter

The effect of treatment and block on stem diameter were not found significant (Table 4.5.1.). However, their interaction was significant (P<0.004).

The average stem diameter for fertilizer treatment T2 was the highest with 25.95 mm among other treatments. Control T1 was the second with 25.56 mm and third treatment of microbial and chemical fertilizer T3 was found the lowest with 24.60 mm on average stem diameter (Table 4.5.2.).

Table 4.5.1. Analysis of variance for the effect of microbial fertilizer on average stem diameter (mm) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	19,139	2	9,569	1,501	,233
Block	14,233	3	4,744	,744	,531
Treatment * Block	141,245	6	23,541	3,692	,004
Error	306,035	48	6,376		
Total	39100,895	60			

Table 4.5.2. The effect of microbial fertilizer on average stem diameter (mm) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	**B**	**C**	**D**	**Average (mm)**
T1- Control	23,71	25,35	25,08	28,10	25,56
T2- Chemical Fertilizer	24,58	27,33	28,08	23,81	25,95
T3- Microbial + Chemical Fertilizer	25,73	24,12	22,50	26,07	24,60
Average	24,67	25,60	25,22	25,99	

*Standard Error for Treatments = 0,7985, **Standard Error for Blocks = 0,9220

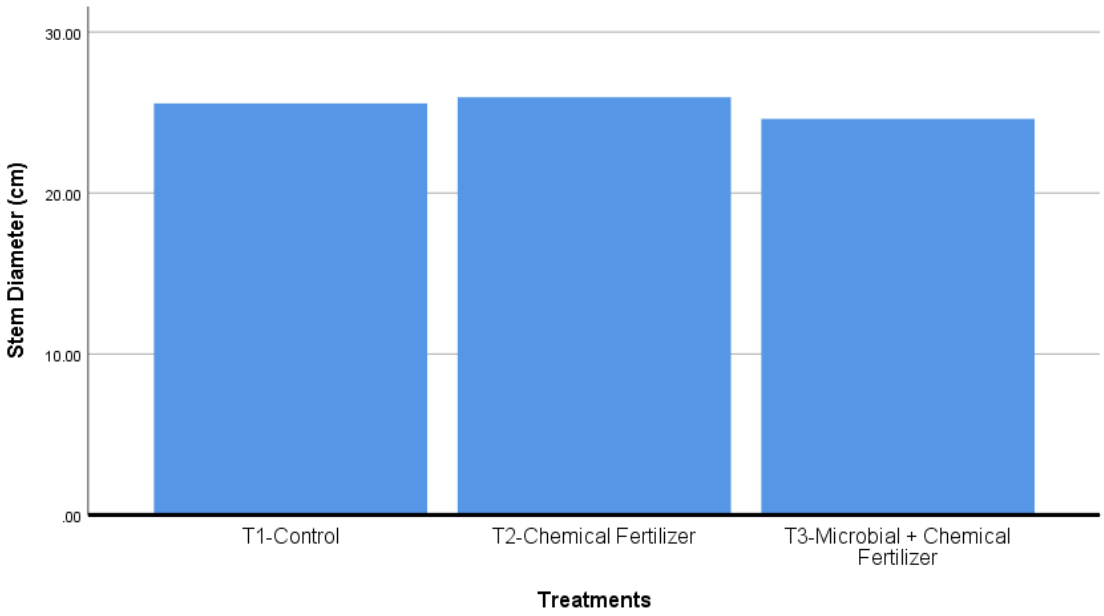

Figure 4.5. The effect of microbial fertilizer on average stem diameter (mm) of green leaf lettuce variety (Funly) under greenhouse conditions

4.6. Head Height

The effect of blocks was found significant (P<0.006) on head height (Table 4.6.1.). Treatment and treatment*block interaction were not significant.

The average head height for fertilizer treatment T2 was the highest with 16.00 cm among other treatments. Microbial and chemical fertilizer treatment T3 was the second with 15.83 cm and Control T1 was found the lowest with 15.55 cm on average head height (Table 4.6.2.).

Table 4.6.1. Analysis of variance for the effect of microbial fertilizer on average head height (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	2,058	2	1,029	,481	,621
Block	29,846	3	9,949	4,654	,006
Treatment * Block	15,642	6	2,607	1,220	,313
Error	102,600	48	2,138		
Total	15112,750	60			

Table 4.6.2. The effect of microbial fertilizer on average head height (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	B	C	D	Average (cm)
T1- Control	15,20	14,80	15,30	16,90	15,55
T2- Chemical Fertilizer	16,70	15,40	15,60	16,30	16,00
T3- Microbial + Chemical Fertilizer	16,50	15,70	14,00	17,10	15,83
Average	16,13 bc	15,30 ab	14,97 a	16,40 c	

*Standard Error for Treatments = 0,4623, **Standard Error for Blocks = 0,5339

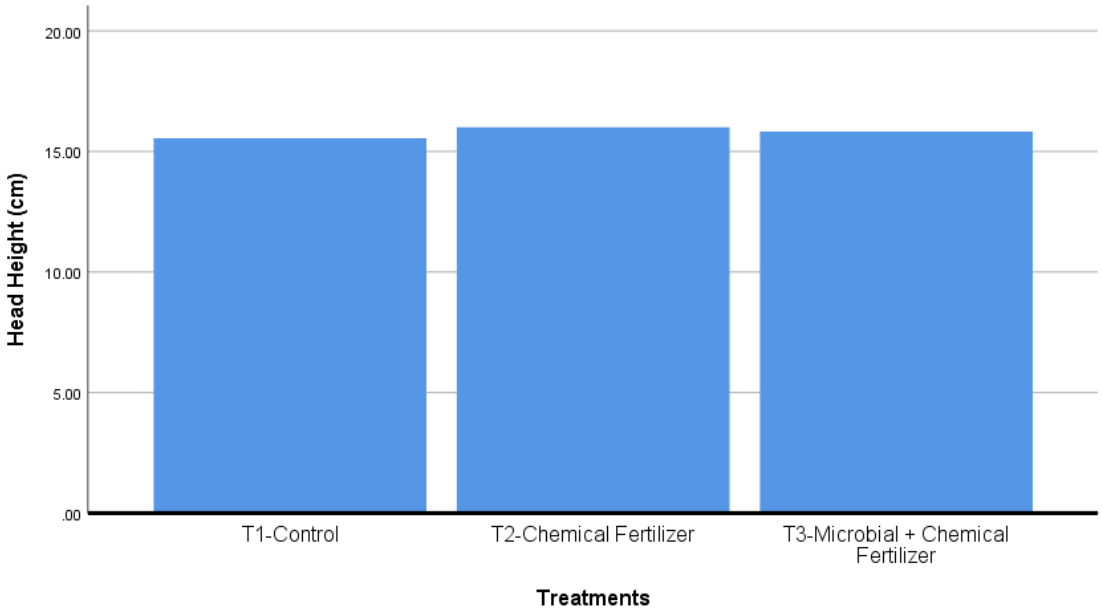

Figure 4.6. The effect of microbial fertilizer on average head height (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

4.7. Head Diameter

The effect of treatment and blocks were not found significant on head diameter (Table 4.7.1.). However, their interaction was found significant (P<0.014).

The average plant diameter for fertilizer treatment T2 was the highest with 81.80 cm among other treatments. Microbial and chemical fertilizer treatment T3 was the second with 81.70 cm and Control T1 was found the lowest with 79.45 cm on average plant diameter (Table 4.7).

Table 4.7.1. Analysis of variance for the effect of microbial fertilizer on average head diameter (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	70,633	2	35,317	,529	,592
Block	144. ,450	3	48,150	,722	,544
Treatment * Block	1207,500	6	201,250	3,016	,014
Error	3202,400	48	66,717		
Total	398123,000	60			

Table 4.7.2. The effect of microbial fertilizer on average head diameter (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	**Blok**				
	A	**B**	**C**	**D**	**Average (cm)**
T1- Control	75,80	82,80	78,60	80,60	79,45
T2- Chemical Fertilizer	75,80	86,60	89,20	75,60	81,80
T3- Microbial + Chemical Fertilizer	83,60	78,20	76,40	88,60	81,70
Average	78,40	82,53	81,40	81,60	

*Standard Error for Treatments = 2,5830, **Standard Error for Blocks = 2,9825

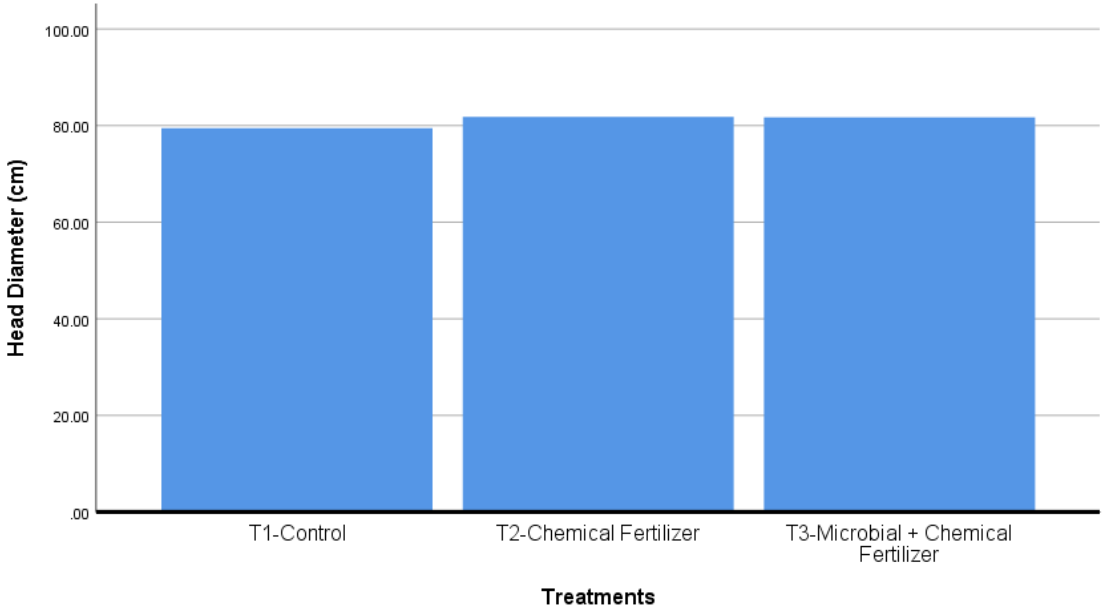

Figure 4.7. The effect of microbial fertilizer on average head diameter (cm) of green leaf lettuce variety (Funly) under greenhouse conditions

4.8. Number of Leaves

The average number of leaves for microbial and chemical fertilizer treatment T3 was the highest with 44 pcs among other treatments. Control T1 was the second with 42.25 pcs and fertilizer treatment T2 was found the lowest with 41.75 pcs on average number of leaves (Table 4.8.2.).

Table 4.8.1. Analysis of variance for the effect of microbial fertilizer on average number of leaves (pcs) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	11,167	2	5,583	,395	,69
Block	64,667	3	21,556	1,525	,302
Treatment * Block	84,833	6	14,139	.	.
Error	,000	0	.		
Total	22006,000	12			

Table 4.8.2. The effect of microbial fertilizer on average number of leaves (pcs) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	B	C	D	Average (pcs)
T1- Control	45,00	39,00	42,00	43,00	42,25
T2- Chemical Fertilizer	46,00	38,00	45,00	38,00	41,75
T3- Microbial + Chemical Fertilizer	49,00	47,00	38,00	42,00	44,00
Average	46,67	41,33	41,67	41,00	

*Standard Error for Treatments = 2,6590, **Standard Error for Blocks = 3,0700

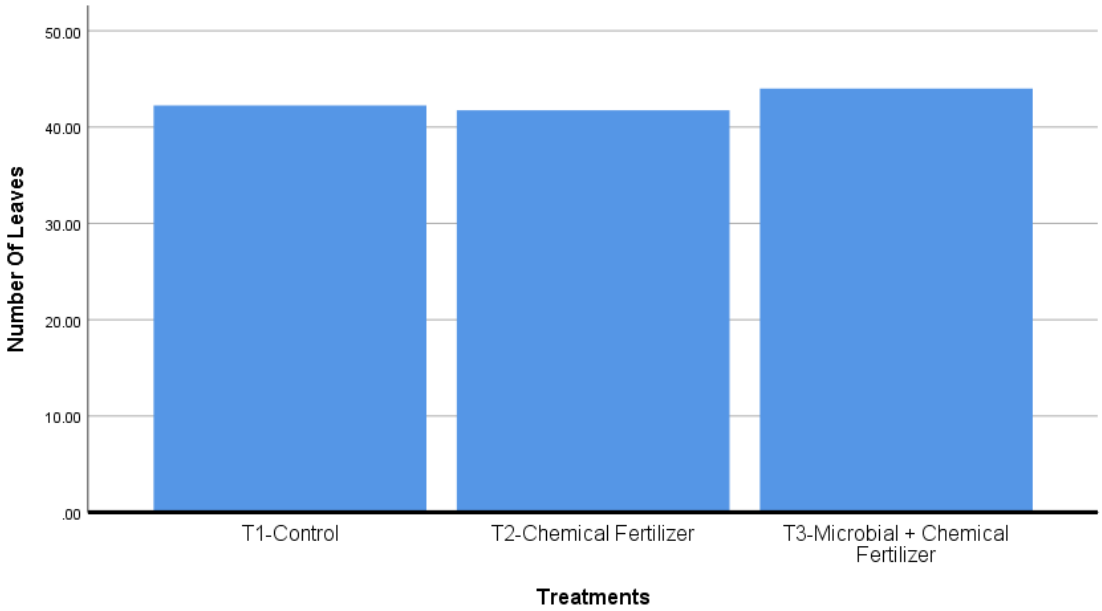

Figure 4.8. The effect of microbial fertilizer on average number of leaves (pcs) of green leaf lettuce variety (Funly) under greenhouse conditions

4.9. Fresh Weight of Leaves

The effect of treatment on fresh weight of leaves was not found significant. (Table 4.9.1.). The effect of blocks (P<0.049) and treatment*block interaction (P<0.002) were found significant.

The average fresh weight of leaves for microbial and chemical fertilizer treatment T3 was the highest with 390.64 g/plant among other treatments. Control T1 was the second with 372.85 g/plant and third treatment of Fertilizer T2 was found the lowest with 369.73 g/plant on average fresh weight of leaves (Table 4.9.2.).

Table 4.9.1. Analysis of variance for the effect of microbial fertilizer on average fresh weight of leaves (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	5091,246	2	2545,623	,338	,715
Block	63373,218	3	21124,406	2,807	,049
Treatment * Block	193762,302	6	32293,717	4,291	,002
Error	361232,896	48	7525,685		
Total	9184634,570	60			

Table 4.9.2. The effect of microbial fertilizer on average fresh weight of leaves (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	**B**	**C**	**D**	**Average (g/plant)**
T1- Control	344,74	351,02	385,18	410,46	372,85
T2- Chemical Fertilizer	414,04	378,52	380,14	306,20	369,73
T3- Microbial + Chemical Fertilizer	507,82	383,66	229,34	441,74	390,64
Average	422,20 b	371,07 ab	331,55 a	386,13 ab	

*Standard Error for Treatments = 27,4330, **Standard Error for Blocks = 31,6769

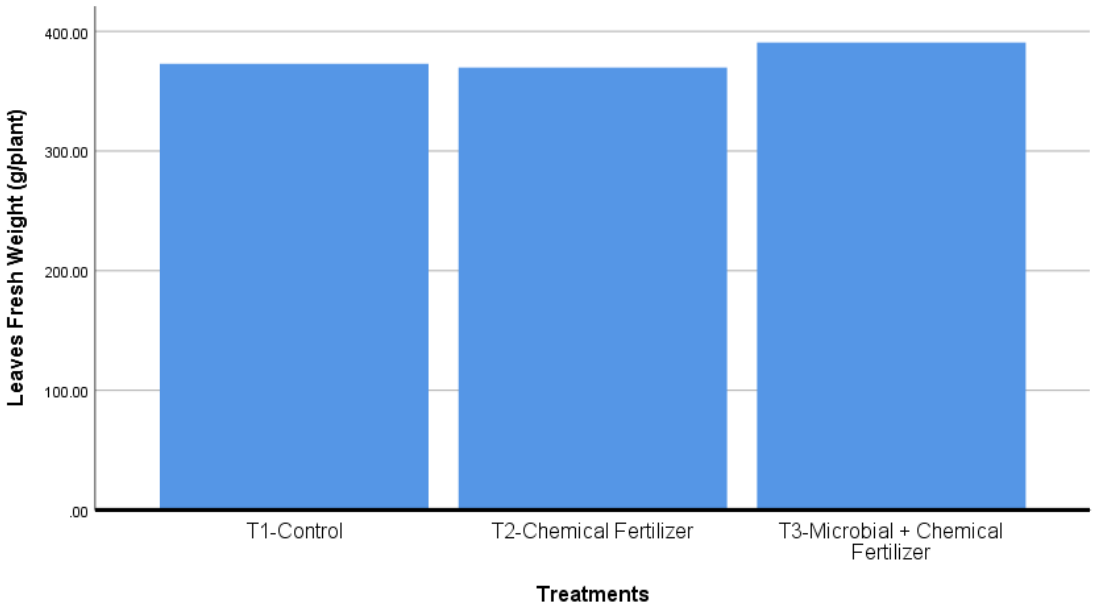

Figure 4.9. The effect of microbial fertilizer on average fresh weight of leaves (g/plant) of green leaf lettuce variety (Funly) under greenhouse conditions

4.10. Dry Matter Percentage of Leaves

The effect of blocks was found significant (P<0.041) on dry matter percentage of leaves (Table 4.10.1.). The effect of treatment and treatment*block interactions were not found significant.

The average dry matter percentage of leaves for microbial and chemical fertilizer treatment T3 was the highest with 19.66 % among other treatments. Control T1 was the second with 19.33 % and third treatment of Fertilizer T2 was found the lowest with 18.17 % on average dry matter percentage of leaves (Table 4.10.2.).

Table 4.10.1. Analysis of variance for the effect of microbial fertilizer on dry matter percentage of leaves (%) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	31,033	2	15,517	1,427	,250
Block	96,583	3	32,194	2,960	,041
Treatment * Block	41,367	6	6894	,634	,702
Error	522,000	48	10,875		
Total	22313,000	60			

Table 4.10.2. The effect of microbial fertilizer on dry matter percentage of leaves (%) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	**B**	**C**	**D**	**Average (%)**
T1- Control	21,36	20,19	17,93	17,82	19,33
T2- Chemical Fertilizer	17,83	18,99	17,51	18,34	18,17
T3- Microbial + Chemical Fertilizer	20,85	22,49	18,03	17,29	19,66
Average	20,01 ab	20,56 b	17,82 a	17,82 a	

*Standard Error for Treatments = 1,0428, **Standard Error for Blocks = 1,2042

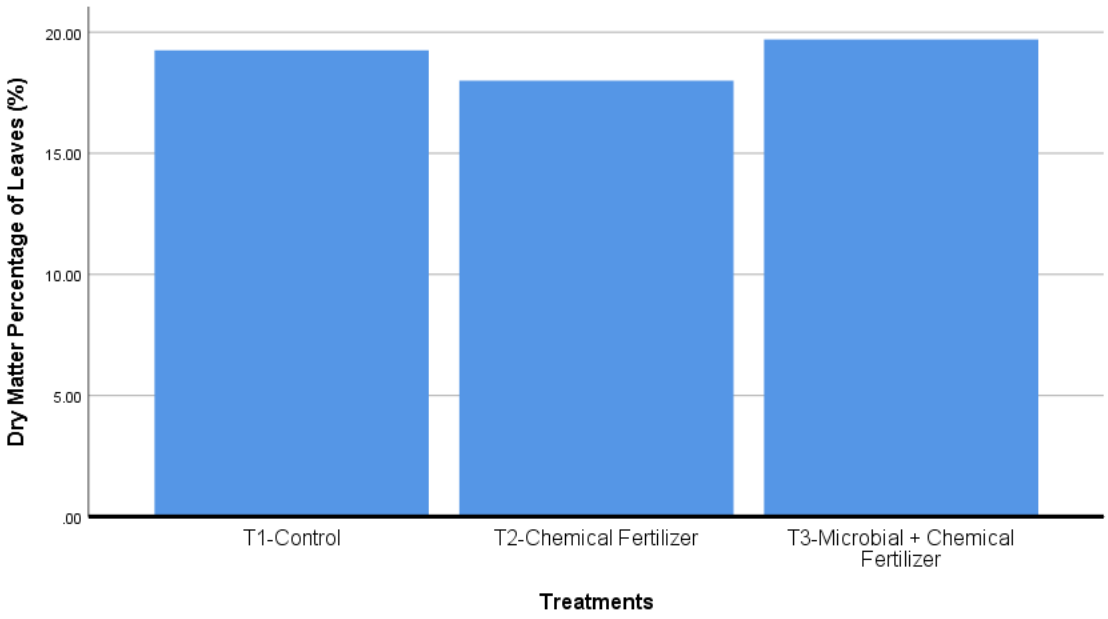

Figure 4.10. The effect of microbial fertilizer on dry matter percentage of leaves (%) of green leaf lettuce variety (Funly) under greenhouse conditions

4.11. Brix Value

The effect of blocks was significant (P<0.039) on brix value (Table 4.11.1.). However, treatment and treatment*block interaction were insignificant.

The average Brix level for Control T1 and fertilizer treatment T2 were the same 2.29 %. Microbial and chemical fertilizer treatment T3 was found the lowest with 2.08 % on average Brix level (Table 4.11.2.).

Table 4.11.1. Analysis of variance for the effect of microbial fertilizer on average brix (%) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	,347	2	,174	1,136	,338
Block	1,500	3	,500	3,273	,039
Treatment * Block	,708	6	,118	,773	,599
Error	3,667	24	,153		
Total	184,000	36			

Table 4.11.2. The effect of microbial fertilizer on average brix (%) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	**B**	**C**	**D**	**Average (%)**
T1- Control	2,50	2,33	2,00	2,33	2,29
T2- Chemical Fertilizer	2,50	2,50	1,83	2,33	2,29
T3- Microbial + Chemical Fertilizer	2,33	2,33	2,00	1,67	2,08
Average	2,44 b	2,39 b	1,94 a	2,11 ab	

*Standard Error for Treatments = 0,1596, **Standard Error for Blocks = 0,1843

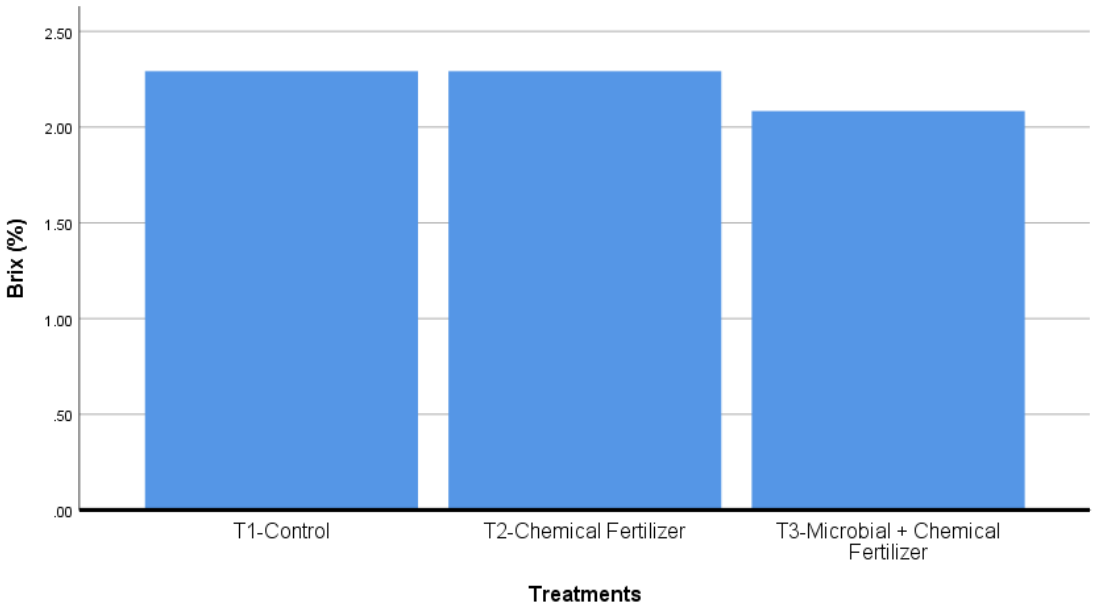

Figure 4.11. The effect of microbial fertilizer on average brix (%) of green leaf lettuce variety (Funly) under greenhouse conditions

4.12. Chlorophyll Content

The effect of blocks was found significant (P<0.000) on chlorophyll content (Table 4.12.1.). Treatment and treatment*block interaction were not significant.

The average chlorophyll compound for control T1 was the highest with 25,56 among other treatments. Fertilizer treatment T2 was the second with 24,67 and third treatment of microbial and chemical fertilizer T3 was found the lowest with 23,82 on average chlorophyll compound (Table 4.12.2.).

Table 4.12.1. Analysis of variance for the effect of microbial fertilizer on average chlorophyll compound (SPAD)of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	61,451	2	30,726	1,902	,154
Block	447,096	3	149,032	9,224	,000
Treatment * Block	139,426	6	23,238	1,438	,207
Error	1745,010	108	16,158		
Total	75534,640	120			

Table 4.12.2. The effect of microbial fertilizer on average chlorophyll compound (SPAD) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	**Blok**				
	A	**B**	**C**	**D**	**Average (SPAD)**
T1- Control	30,29	26,72	23,73	21,56	25,58
T2- Chemical Fertilizer	25,44	26,37	24,1	22,76	24,67
T3- Microbial + Chemical Fertilizer	25,74	24,63	22,25	22,67	23,82
Average	27,16 b	25,91 b	23,36 a	22,33 a	

*Standard Error for Treatments = 0,8988, **Standard Error for Blocks = 1,0379

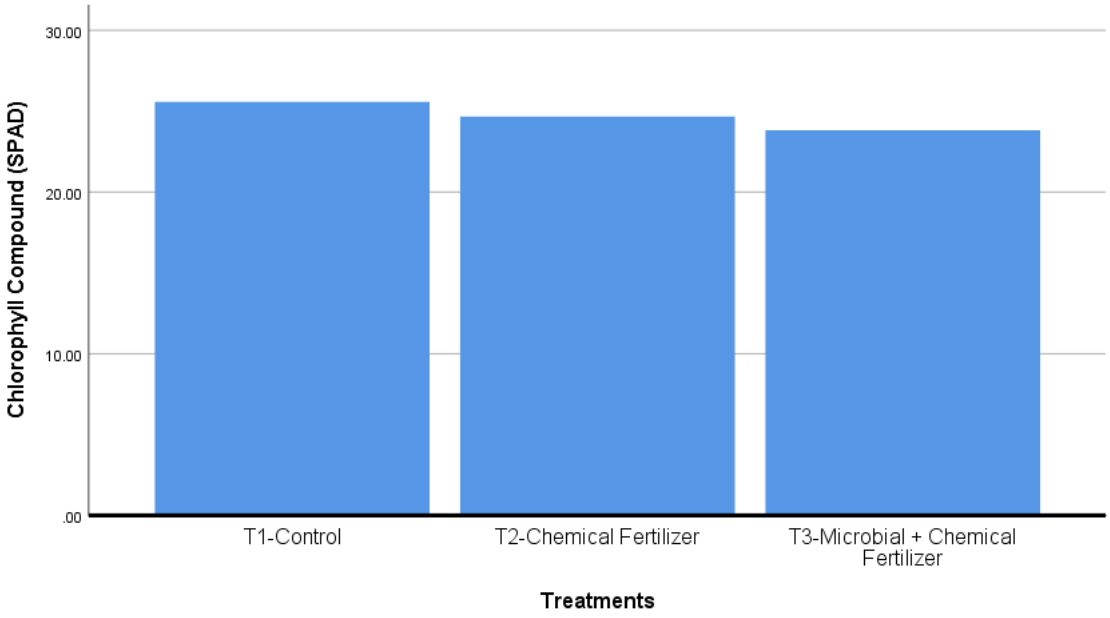

Figure 4.12. The effect of microbial fertilizer on average chlorophyll compound (SPAD) of green leaf lettuce variety (Funly) under greenhouse conditions

4.13. Leaf Color

The effect of treatment (P<0.011) and blocks (P<0.000) were found significant on leaf color (Table 4.13.1.). Their interaction was not found significant.

As a measure of green color "b" values were used. Control T1 has the highest value with 39,10 among other treatments. Fertilizer treatment T2 was the second with 38,18 and third treatment of microbial and chemical fertilizer T3 was found the lowest with a value of 37,71 for green color (Table 4.13.2.).

Table 4.13.1. Analysis of variance for the effect of microbial fertilizer on leaf color of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	40,066	2	20,033	4,694	,011
Block	163,123	3	54,374	12,741	,000
Treatment * Block	50,651	6	8,442	1,978	,075
Error	460,900	108	4,268		
Total	177012,807	120			

Table 4.13.2. The effect of microbial fertilizer on leaf color of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	**B**	**C**	**D**	**Average (b*)**
T1- Control	36,50	38,40	39,87	41,63	39,10 b
T2- Chemical Fertilizer	37,18	36,94	39,22	39,37	38,18 a
T3- Microbial + Chemical Fertilizer	36,51	37,86	38,46	38,01	37,71 a
Average	36,73 a	37,73 a	39,18 b	39,67 b	

*Standard Error for Treatments = 0,4619, **Standard Error for Blocks = 0,5333

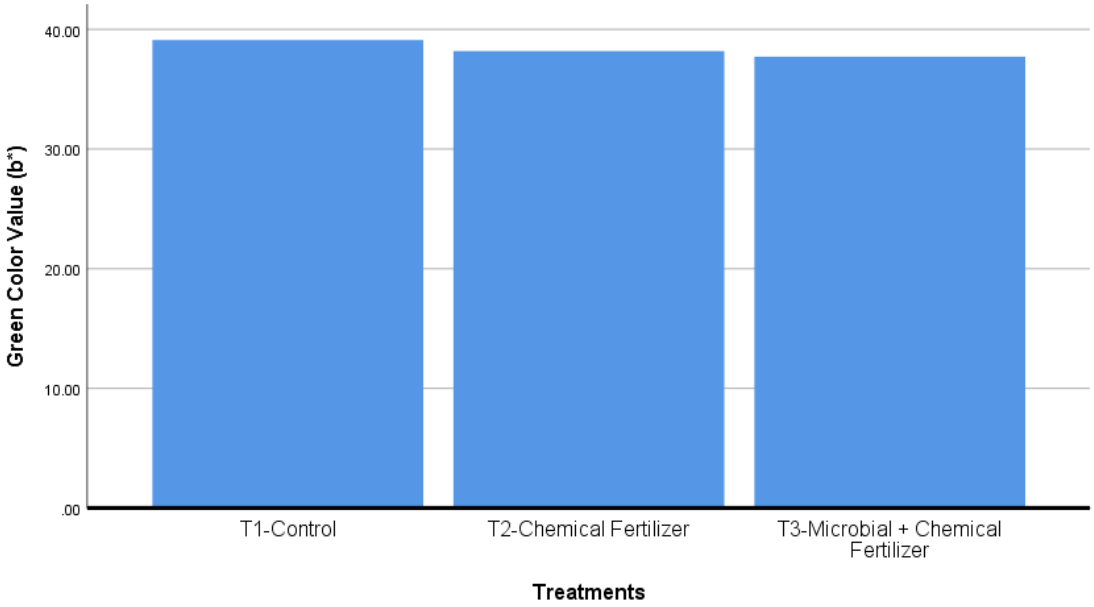

Figure 4.13. The effect of microbial fertilizer on leaf color of green leaf lettuce variety (Funly) under greenhouse conditions

4.14. Marketable Head Yield

The effect of treatment on marketable head yield was found significant but, the effect of blocks was not significant (Table 4.14.1.).

Marketable head yield for Microbial + Chemical Fertilizer treatment T3 was highest with 2808,378 kg. Chemical Fertilizer treatment T2 has the second highest yield with 2528,378 kg. Control T1 treatment has the lowest yield of 2330,313 kg. (Table 4.14).

Table 4.14.1. Analysis of variance for the effect of microbial fertilizer on marketable head yield (kg/decare) of green leaf lettuce variety (Funly) under greenhouse conditions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	461956,167	2	230978,083	4,820	,056
Block	253611,333	3	84537,111	1,764	,254
Treatment * Block					
Error	287549,167	6	47924,861		
Total	461956,167	2	230978,083	4,820	,056

Table 4.14.2. The effect of microbial fertilizer on marketable head yield (kg/decare) of green leaf lettuce variety (Funly) under greenhouse conditions

Treatment	Blok				
	A	B	C	D	Average (kg/decare)
T1- Control	2154,63	2193,88	2407,38	2565,38	2330,313 a
T2- Chemical Fertilizer	2587,75	2365,75	2375,88	2783,43	2528,202 ab
T3- Microbial + Chemical Fertilizer	3173,88	2397,88	2900,89	2760,88	2808,378 b
Average	2638,75	2319,17	2561,38	2703,23	

*Standard Error for Treatments = 154,7980, **Standard Error for Blocks = 178,7450

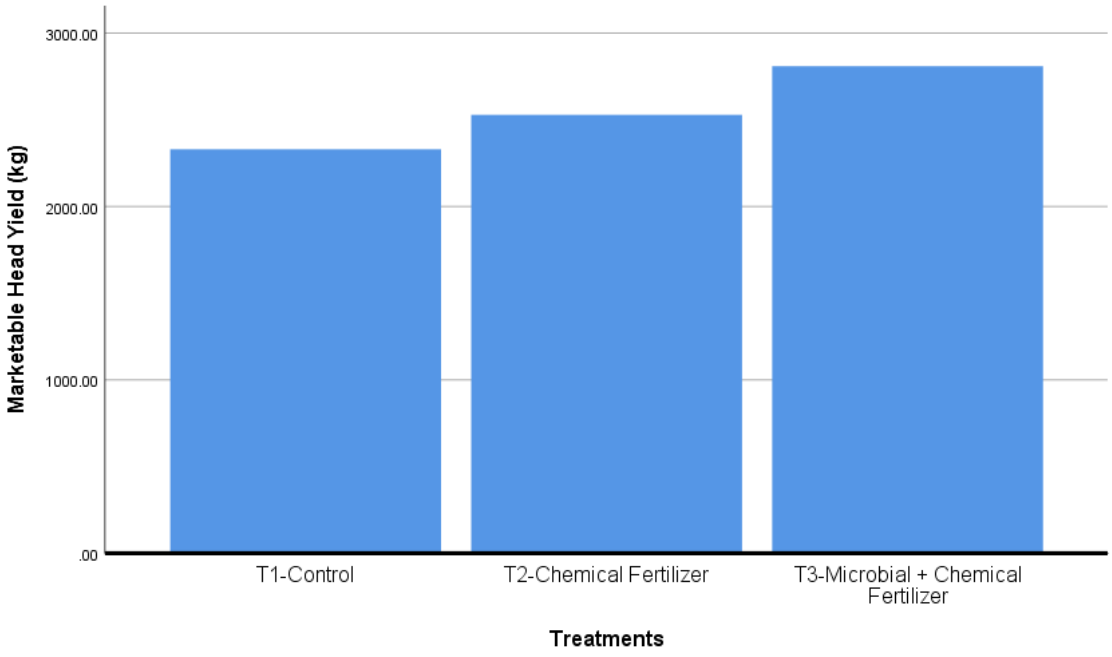

Figure 4.14. The effect of microbial fertilizer on marketable head yield (kg/decare) of green leaf lettuce variety (Funly) under greenhouse conditions

4.15. Correlation Analysis

Correlation analysis has been done between plant growth parameters measured (Table 4.15.).

Table 4.15. Correlation analysis between plant growth parameters

	Root Length	Root Collar Diameter	Root Fresh Weight	Root Dry Weight	Stem Diameter	Head Height	Head Diameter	Leaves Fresh Weight	Dry Matter Percentage	Brix	SPAD Value	Leaf Color	Yield
Root Length	1												
Root Collar Diameter	0,029	1											
Root Fresh Weight	,497**	,261*	1										
Root Dry Weight	,443**	,366**	,884**	1									
Stem Diameter	,275*	,755**	,308*	,437**	1								
Head Height	-0,161	,426**	-0,065	-0,111	,427**	1							
Head Diameter	0,252	,566**	,399**	,499**	,792**	,290*	1						
Leaves Fresh Weight	0,158	,617**	,369**	,381**	,752**	,590**	,690**	1					
Dry Matter Percentage	-0,038	-,444**	0,049	-0,037	-,572**	-0,188	-,557**	-,317*	1				
Brix	0,162	-0,184	0,059	-0,037	0,037	0,037	-0,187	0,030	-0,062	1			
SPAD Value	0,159	-0,012	0,217	0,203	-0,049	-0,148	-0,109	0,017	0,208	0,299	1		
Leaf Color	-0,014	,272*	-0,143	-0,173	0,164	0,153	0,084	-0,004	-0,251	-0,125	-,625**	1	
Yield	-0,281	0,015	0,025	0,250	0,282	,664*	0,483	0,502	0,115	0,140	-0,181	0,094	1

*values with * are significant at the probability level (P<0,05), ** values with * *are significant at the probability level (P<0,01)

4.16. Discussion

In this study, the possible use of a bacterial mixture of *Bacillus subtilis* VKPM B-10641 (DSM 24613) and *Bacillus amyloliquefaciens* VKPM B-10642 (DSM 24614) and B-10643 (DSM 24615) in equal ratios as biological fertilizer in conjunction with mineral fertilizers was investigated with an aim of investigating the effect on biomass production and chlorophyll content of lettuce by increasing nutrient intake from the soil.

The effect on biomass production was evaluated by means of commercial yield and other plant growth traits that determine commercial yield. The traits measured were root length, root collar diameter, root fresh weight, root dry weight, stem diameter, head diameter, head height, number of leaves, fresh weight of leaves, dry matter percentage of leaves, brix value, and leaf color.

Use of microbial fertilizer in conjunction with chemical fertilizer decreased root length of lettuce in comparison to control and chemical fertilizer application. Even if it was not found significant, the root length was 14,7 ± 0,64 cm being 5 % less than control (15,5 ± 0,64 c and 1 % less than chemical fertilizer application (14,8 ± 0,64 cm). Use of chemical and microbial fertilizers in combination probably increased efficient intake of nutrients by the roots and consequently longer and deeper root development was not needed to reach deeper nutrients in the soil. As there was a decrease on root length, root collar diameter was also affected in the same direction since there was shorter root formation. Root collar diameter was 20,97 ± 0,81 mm in combined application being 7 % less than control (22,63 ± 0,81 mm) and 1 % less than chemical fertilizer application (21,66 ± 0,81 mm). However, it was found opposite in some other studies done on *Arabidopsis* plant, radish and lettuce where primary root length, lateral root architecture was improved in the presence of plant growth promoting bacteria (Poitout et al. 2017 and Hong and Lee 2017).

It was found that there is a correlation between root length, root diameter and root fresh weight, however root fresh weight was 20,77 ± 1,05 g/plant being 6 % higher than chemical fertilizer application (19,58 ± 1,05 g/plant) in microbial and chemical fertilizer application respectively, but it was 3 % less than control 21,33 ± 1,05 g/plant. However, the treatment effect was not significant for dry weight of roots, it was in parallel with root length and root collar diameter which was highly correlated. Root dry weight was the highest with 7,35 ± 0,11 g/plant in control, 7,32 ± 0,11 g/plant in chemical fertilizer application and lowest in microbial and chemical fertilizer application with 7,29 ± 0,11 g/plant. According to Barazani and Friedman, benefits of PGPR strains dependent on the amount of auxins and indole acetic acids they produce and root development can be promoted or suppressed (Barazani and Friedman 1999).

However, the effect of treatments was not significant for stem development, stem diameter was the lowest in microbial and chemical fertilizer application with 24,60 ± 0,80 mm which was 4 % less than control 25,56 ± 0,80 mm and 5 % less than chemical fertilizer application 25,95 ± 0,80 mm. Stem diameter and head height were significantly highly correlated ($P<0.01$). Since the effect of treatments were not significant for stem diameter, control was the lowest with 15,55 ± 0,46 mm and chemical fertilizer application was the highest with 16,00 ± 0,46 mm. Microbial and chemical fertilizer application was 1 % less than chemical fertilizer application and 2 % higher than control with 15,83 ±

0,46 mm head height. Head diameter was also affected in the same way with height of the head and the effect of treatments on head diameter were not significant. Head diameter was the highest with 81,80 ± 2,58 mm in chemical fertilizer application, and it was also close the value of 81,70 ± 2,58 mm in microbial and chemical fertilizer application. Control was the lowest with 3 % smaller head diameter of 79,45 ± 2,58 mm compared to other treatments.

Number of leaves, fresh weight and dry matter percentage of leaves were higher in the application of microbial and chemical fertilizer combination. However, our results were not statistically significant. Average number of in microbial and chemical fertilizer application was 4 % higher (44,00 ± 2,66 leaf/plant) over control (42,25 ± 2,66 leaf/plant) and 5 % higher over chemical fertilizer application (41,75 ± 2,66 leaf/plant). As there was higher number of leaves in microbial and chemical fertilizer application, average fresh weight of leaves per plant was also higher (390,64 ± 27,43 g/plant). It was 5 % higher over control (372,85 ± 27,43 g/plant) and 6 % higher over chemical fertilizer (369,73 ± 27,43 g/plant). Dry matter percentage was affected in the same way and the effect was not statistically significant. but, the difference was much higher than number of leaves and fresh weight of leaves per plant. Dry matter was 19,66 ± 1,04 % in microbial and chemical fertilizer treatment being 2 % more than control (19,33 ± 1,04 %) and 9 % more than chemical fertilizer treatment (18,17 ± 1,04 %). On the contrary brix level in microbial and chemical fertilizer treatment was 9 % lower (2,08 ± 0,16 %) than control and chemical fertilizer treatments (2,29 ± 0,16 % for both). As it was reported by Souza et al. (2015) and Vejan et al. (2016), PGRP enhanced plant growth via various mechanisms and has the potential to be an agriculturally beneficial microbe for stimulating plant development. In another study done with different PGPR strains on Arabidopsis and lettuce, microbial fertilizer application facilitated the growth of plants through numerous means, including through the induction of cell development, nitrate transport, and metabolic stimulation (Trinh et al. 2018).

Even though the effect of treatments on chlorophyll content was not significant, it was highly correlated with green color of leaves which was significant ($P<0.011$). Chlorophyll content (SPAD) was the lowest in application of microbial and chemical fertilizer combination (23,82 ± 0,90) by 7 % less compared to control (25,58 ± 0,90) and by 4 % less compared to chemical fertilizer treatment (24,67 ± 0,90). b* Value of leaf color was also in the same direction being 4 % less in microbial and chemical fertilizer treatment (37,71 ± 0,46) compared to control (39,10 ± 0,46) and 1 % less compared to chemical fertilizer treatment (38,18 ± 0,46). Chlorophyll content was affected by many environmental factors including type of plant and position of leaves (Gond et al. 2012). Karakurt et al. (2009) reports in a study on cherry that PGPR applications had an effect on color and brightness. PGPR was previously shown to exert beneficial effects on plant development and stimulated the yield and quality parameters of sugar beet, barley (Cakmakci et al. 2001) and raspberry (Orhan et al. 2006) in the field via direct or indirect mechanisms. PGPR-mediated increase in the availability of nutrients in the rhizosphere was proposed as the mechanism by which PGPR enhanced the crop yield and increased the fruit size (Bar-Ness et al. 1992; Richardson 2001). Kim et al. (2017) when the plants were adequately supplied with all the nutrients, observed positive growth effect possibly by hormone production.

It was also found that there was a correlation between head height and marketable head yield. However, there was only head height in correlation with total yield. Number and dry matter percentage of leaves probably affected total yield also. Lucy et al. (2004) also reported that PGPRs were beneficial for leaf area and content. As a combined effect of all traits on commercial yield, microbial and chemical fertilizer application gave the highest yield. Total yield of microbial and chemical fertilizer applied blocks were 20 % higher (2808,38 ± 154,80 kg/da) than control treatment (2330,31 ± 154,80 kg/da) and 11 % higher than chemical fertilizer treatment (2528,20 ± 154,80 kg/da). Althoughit was not significant in analysis of variance, there was significant difference according to Duncan analysis. Cipriano et al. (2016) also reported that two strains with different plant growth promoting traits, including phosphate solubilization, hormone production, and antagonism to pathogen compounds, improved lettuce plant biomass yields up to 30%, in a study to evaluate the effect of 54 *Pseudomonas* strains on lettuce growth. According to the increase rates by chemical fertilizer application alone and microbial and chemical fertilizer combination, it was suggested to use microbial fertilizers in combination with chemical fertilizers. Combined application may give better results in warmer seasons with higher soil temperatures.

6. CONCLUSIONS

In this study, the effect of *Bacillus subtilis* and *Bacillus amyloliquefaciens* mixture on biomass production and chlorophyll content of lettuce (*Lactuca sativa*) were investigated in spring 2015. Our results showed that application of bacterial and chemical fertilizer in combination produced higher total marketable head yields than untreated blocks. The yield was increased by having 4 % higher number of leaves, 2 % taller head height, 3 % bigger head diameter and 2 % higher dry matter concentration of leaves.

The combined application *Bacillus subtilis* and *Bacillus amyloliquefaciens* we achieved 20 % more yield compared to control and 11 % more yield compared to standard chemical fertilizer application. According to our experiment chemical fertilization alone was not enough to increase marketable head yield more than 8 %. As Kim et al. (2017) suggest that, even the plants were adequately supplied with all the nutrients, the observed positive growth effect might be affected by hormone production. Microbial fertilizer application probably increased the efficiency of chemical fertilizers and induced plant growth by their hormone production. This increase in yield was statistically significant and it is an important rate for growers in terms of commercial yield. Microbial fertilizers can thus be suggested for growers to increase the effect of chemical fertilizers.

Further experiments are suggested with different concentration of chemical and microbial fertilizer applications and should be repeated in other seasons with different soil conditions to measure other benefits of this microbial fertilizer on seed germination, harmful rhizosphere microorganisms, suppressing and protecting against bacterial, fungal, nematode and viral diseases.

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