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The Effect of *Azotobacter chrococcum* as Nitrogen biofertilizer on the growth and yield of *Cucumis Sativus*

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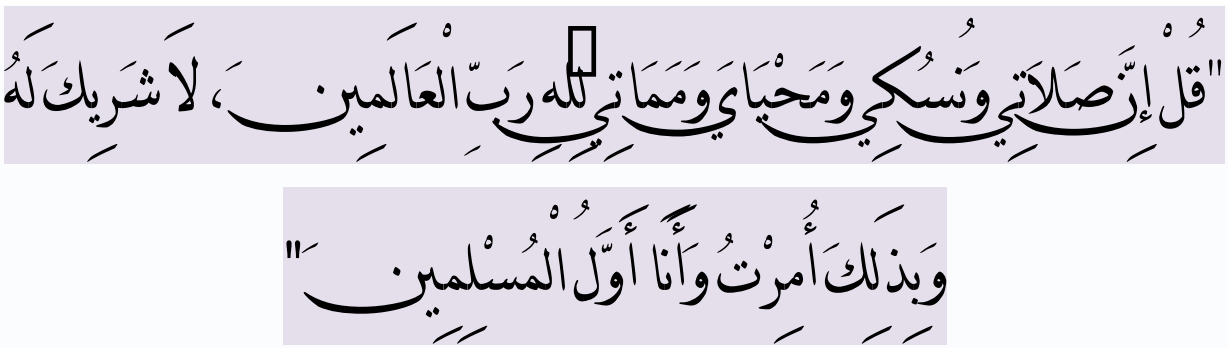

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university of other institute, except where due acknowledgment has been made in the text .

Signature	**Name**	**Date**
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DEDICATION

To my parents for their continued support

To my wife who helped me to accomplish this thesis

TO my brother hassan

To my brothers and sisters

To my university "IUG"

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Alhamdulillah. I am very grateful to **Allah s.w.t.** for giving me patience, guidance and strength which enabled me to successfully complete my research.

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The effect of *Azotobacter chroococcum* as nitrogen biofertilizer on the growth and yield of *Cucumis sativus*

Abstract

Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. The use of biofertilizer is steadily increased in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. The main objective of this study is to evaluate the effect of *Azotobacter chroococcum* as nitrogen-biofertilizer on growth and yield of ***Cucumis sativus* (cucumber)** under greenhouse conditions. The study was done by planting 210 cucumber seeds distributed into seven treatments which were used in our study as follows: control (without treatment), biofertilizer only, organic fertilizer only, chemical fertilizer only, organic fertilizer + biofertilizer, 20% chemical fertilizer + biofertilizer, and biofertilizer, (two dose). After 3 months and through **cucumber** growth criteria, (shoot length, root length, shoot wet and dry weight, root wet and dry weight, number of leaves, number of branches), yield parameters, mineral content (N%) of **cucumber** were measured. In the green house experiment, growth parameters of **cucumber** showed that the productivity of cucumber increased. Seed inoculation with *A. chroococcum* increased yield about 6%, compared to control. The increase of biofertilizer treated plants in dry root weight were 31%, 18% in wet root weight, 11% in dry whole plant weight, 13% in wet whole plant weight, 14% in whole plant length, 10% in number of branches, 27% in number of leaves over control. The increase in shoot nitrogen percentage was 15% in biofertilizer treated plants, where it was 40% in biofertilizer + 20% chemical over control. The increase in root nitrogen percentage was 18% in biofertilizer treated plants, where it was 22% in biofertilizer + 20% chemical over control. Our results provided a proof of the efficiency of *Azotobacter chroococcum* as an important biofertilizer in yield of ***Cucumis sativus* (cucumber).**

Key words: *Azotobacter chroococcum,* non symbiotic nitrogen fixation, cucumber, biofertilizer, chemical fertilizer.

فحص تأثير *Azotobacter chroococcum* كسماد حيوي على نبات الخيار

المستخلص

يعتبر التسميد الحيوي عنصر هام من عناصر تقليل الضرر الناتج عن استخدام الأسمدة الكيماوية ويسد جزء كبير من الاحتياجات السمادية ويوفر القدر الكبير الذي ينفق في إنتاجها ويساعد على تقليل الطاقة المستخدمة في إنتاجها. ويعتبر الهدف الأساسي من هذه الدراسة هو فحص تأثير استخدام البكتيريا التي تقوم بتثبيت النيتروجين من الجو كسماد حيوي و قد تم التركيز على نوع معين من البكتيريا و هي *A. chroococcum*. لقد تم زراعة 210 من بذور الخيار قسمت على سبع معاملات كل معاملة لها 30 بذرة و كانت المعاملات كالتالي: المعاملة الأولى بدون أي إضافات و الثانية إضافة البكتيريا والثالثة إضافة مخصب عضوي والرابعة إضافة مخصب كيميائي و الخامسة إضافة مخصب حيوي + عضوي و السادسة مخصب حيوي + 20% كيميائي و السابعة جرعتين بكتيريا. استمرت الزراعة لمدة 3 شهور حيث تم خلالها ملاحظة التغييرات على كل معاملة من المعاملات السبع و متابعة النمو وقياس العديد من المتغيرات مثل طول الساق، طول الجذر، عدد الأوراق،عدد التفرعات، وزن الساق الجاف والرطب، وزن الجذر الجاف و الرطب، نسبة النيتروجين في الساق و في الجذر. ومن خلال النتائج التي تم الحصول عليها وجد أن إنتاج الخيار قد زاد بنسبة 6% في النباتات التي تم معاملته ب *A. chroococcum* مقارنة بالكنترول. وكانت الزيادة في وزن الجذر الجاف بنسبة 31% وبنسبة 18% في وزن الجذر الرطب، وبنسبة 11% في الوزن الكلي الجاف للنبات، و بنسبة 13% في الوزن الكلي الرطب للنبات، وبنسبة 14% في الطول الكلي للنبات، وبنسبة 10% في عدد التفرعات، وبنسبة 27% في عدد الأوراق مقارنة بالكنترول. أما نسبة النيتروجين في الساق فكانت الزيادة بنسبة 15% في النباتات التي تم معاملتها بالمخصب الحيوي و الزيادة بنسبة 40% في النباتات التي تم معاملتها بالمخصب الحيوي + 20% مخصب كيميائي مقارنة بالكنترول، بينما زادت نسبة النيتروجين في الجذر بنسبة 18% في النباتات التي تم معاملتها بالمخصب الحيوي و الزيادة بنسبة 22% في النباتات التي تم معاملتها بالمخصب الحيوي + 20% مخصب كيميائي مقارنة بالكنترول. إن نتائج البحث تثبت مدى كفاءة استخدام *A. chroococcum* كمخصب حيوي.

الكلمات المفتاحية: المخصبات الحيوية، الأسمدة الكيميائية، أزوتوباكتر، نبات الخيار، البكتيريا المثبتة للنيتروجين.

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Abbreviations

PSMS	Phosphate Solubilizing Microorganisms
PSB	Phosphate solubilizing bacteria
PSF	Phosphorus solubilizing fungi
IAA	Indol-3-acetic acid
KSB	Potassium Solubilizing Bacteria
PGPR	Plant Growth Promoting Rhizobacteria
PGPB	Plant growth-promoting bacteria
KSB	Potassium Solubilizing Bacteria
PGRs	Plant Growth Regulators
VAM	Vesicular Arbuscular Mycorrhizae
VA-fungi	Vesicular Arbuscular fungi
EM	Ectomycorrhizae
AM	Endomycorrhizae
BNF	Biological Nitrogen Fixation
A	Control (no inoculation)
B	Biofertilizer only (*A. chroococcum*)
C	Organic only (compost)
D	Chemical fertilizer only.
E	Organic + Biofertilizer (*A. chroococcum*)
F	Biofertilizer + 20% Chemical fertilizer
G	Biofertilizer (two doses of *A. chroococcum*)

Chapter I
Introduction

1.1 Overview

Gaza Strip is an agricultural land but the culture of Gaza Strip is severely hampered by high population density, limited land access, water shortages, the effects of Israeli military operations, and restrictions on labour and trade access across the border (Yassin and Abd Rabou, 2002). Farmers in Gaza Strip support the use of chemical fertilizers to increase their products to meet the needs of the population which can affect the artesian water, as well as soil and human health, however, the blockade often affects the ability of farms to obtain their needs of the chemical fertilizers. So it can be replaced by biofertilizer, which could reduce the damage of chemical fertilizers on ground water, the soil and human health, which can lead to maintain the fertility and health of soil in Gaza Strip land and overcome the problems of chemical fertilizers.

Plants, like all other living things need food for their growth and development, and they require 16 essential elements. Carbon, hydrogen, and oxygen are derived from the atmosphere and water soil. The remaining 13 essential elements (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine) are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers (Al-Khiat, 2006).

They are classified into two categories which are macronutrient and micronutrient depending on the quantity required. NPK (nitrogen, phosphorus, potassium) are primary macronutrients element which are needed in large amounts while copper, boron and iron are example of micronutrients that are needed in only very small amount or micro quantity (Ahmad, 2009). For optimum plant growth, nutrients must be available in sufficient and balanced quantities. Soil contains natural reserves of plant nutrients, but these reserves are largely in forms unavailable to plants, and only a minor portion is released each year through biological activity or chemical processes. This release is too slow to compensate for the removal of nutrients by agricultural production and to meet crop requirements (Jen-Hshuan,,2006). In the soil, the mineral nutrients are dissolved in water and absorbed through a plant’s root.

However, the amounts of nutrients in soil are always unpredictable and not enough for plants growth. As a result, primary nutrients NPK which are utilized in the large amounts by crops are commonly found in blended fertilizers nowadays (Ahmad, 2009).

Based on the production process, the fertilizers can be roughly categorized into three types: chemical, organic and biofertilizer. The use of chemical fertilizer or organic fertilizer has its advantages and disadvantages in the context of nutrient supply, crop growth and environmental quality. The advantages need to be integrated in order to make optimum use of each type of fertilizer and achieve balanced nutrient management for crop growth (Jen-Hshuan, 2006).

Runoff of synthetic fertilizer can enter the waterways, causing water to be polluted and to lose oxygen. Overtime, chemical fertilizers can degrade the quality of the soil by building up toxins or leaching away natural nutrients, making the soil unfit for growing plants. Using too much fertilizer can damage plants by chemically burning roots and leaves. Organic fertilizers are more difficult to use than synthetic fertilizers. Because the nutrients in organic fertilizers can vary, it is more difficult to determine how much should be used. Organic fertilizers take longer to break down in the soil and are much less potent, so if they are not applied in the right amounts at the right time, plants may not get the nutrients they need. They are more expensive and must be applied in larger quantities. It is a constant challenge to minimize the use of chemicals in agriculture.

The intensive land use, including the artificial N-fertilizers, in agriculture causes the acidification of soils due to the harvest or leaching of cations. The indirect effect of soil acidity on the presence and availability of toxic ions, such as aluminum, manganese, or other heavy metals, are generally more important to crop production than the direct effect of acidity on the plants. Impacts of soil acidification decrease the number and activity of useful soil organisms, deficiency of magnesium, calcium may occur, phosphorus may become less available, the solubility of several heavy metals may reach toxic levels, increasing uptake of heavy metals by crop plants may cause serious health problems to animals and humans (Lévai *et al*, 2008).

The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water. These problems can be tackled by use of biofertilizers (Saadatnia & Riahi, 2009). Soil microbes are of great importance in cycling nutrients such as carbon (C), nitrogen (N), phosphorus (P), and sulphur (S). Not only do they control the forms of these elements (e.g. specialized soil bacteria convert ammonium N (NH_4^+) to nitrate (NO_3^-) they can regulate the quantities of N available to plants. Beside their effects on the availability of nutrients the bacterial soil life prevents the uptake of several harmful ions. The use of living bacteria (biofertilizer) accelerates mineralization of organic residues in soil, therefore makes the nutrients more available. At the same time due to effect of living bacteria from biofertilizer, the uptake of heavy metals decreases (Lévai *et al*, 2008).

Biofertilizer is defined as a substance which contains living microorganisms and is known to help with expansion of the root system and better seed germination. The microorganisms containing biofertilizers can be the tools we could change apply of chemical fertilizers. Biofertilizers are products containing living cells of different types of microorganism, which have an ability to convert nutritionally important elements to available form through biological processes. In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yield through environmentally better nutrient supplies (Marianna *et al*, 2005). There is a great interest in establishing novel associations between higher plants and various N2-fixing microorganisms (Al-Khiat, 2006).

For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere. Application of beneficial microbes in agricultural practices started 60 years ago and there is now increasing evidence that these beneficial microbial populations can also enhance plant resistance to adverse environmental stresses, e.g. water and nutrient deficiency and heavy metal contamination (Wua *et al*, 2004).
Biofertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth-promoting microorganisms. Among, biofertilizers benefiting the crop

production are *Azotobacter*, *Azospirillum*, *blue green algae*, *Azolla, P-solubilizing microorganisms*, *mycorrhizae* and *sinorhizobium* (Selvakumar *et al*, 2009). Amongst biofertilizers azotobacter strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots. They have been also reported to improve fertility condition of the soil. Aerobic bacteria belonging to the genus *Azotobacter* represent a diverse group of free-living diazotrophic (with the ability to use N_2 as the sole nitrogen source) microorganisms commonly occurring in soil. The genus *Azotobacter* includes 6 species, with *A. chroococcum* most commonly inhabiting various soils all over the world (Mahato *et al*, 2009).

1.2 Primary Macronutrients

1.2.1 Nitrogen

Although Earth's atmosphere contains 78% nitrogen gas (N2), most organisms cannot directly use this resource due to the stability of the compound. Plants, animals and microorganisms can die of nitrogen deficiency, surrounded by N_2 they cannot use. All organisms use the ammonia (NH_3) form of nitrogen to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing components necessary for life (Lindemann and Glover, 2008, Mikkelsen and Hartz, 2008).

Nitrogen is present in all living organisms, in proteins, nucleic acids and other molecules. It typically makes up around 4% of the dry weight of plant matter. (http://en.wikipedia).

Nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is therefore important in plant growth and production of food and feed. Inadequate supply of available N frequently results in plants that have slow growth, depressed protein levels, poor yield of low quality produce, and inefficient water use (Mikkelsen and Hartz, 2008, Rifat *et al*, 2010).

The sources of nitrogen used in fertilizers are many, including ammonia (NH_3), diammonium phosphate ($(NH_4)_2HPO_4$), ammonium nitrate (NH_4NO_3), ammonium sulfate ($(NH_4)_2SO_4$), calcium cyanamide ($CaCN_2$), calcium nitrate ($Ca(NO_3)_2$), sodium nitrate ($NaNO_3$), and urea (N_2H_4CO) (Shakhashiri, 2003).

1.2.2 Phosphorus

Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Ahmad *et al*, 2009). Although phosphorus uptake by plants is less compared to nitrogen and potassium, normal plant growth cannot be achieved without it (Bin Zakaria, 2009). P in soils is immobilized or becomes less soluble either by absorption, chemical precipitation, or both (Tilak *et al*, 2005). The concentration of soluble phosphorus (P) in tropical soil is usually very low, phosphorus is only available in micromolar quantities or less (Henri *et al,* 2006). The P-content in average soils is about 0.05% (w/w) but only 0.1% of the total P is available to plants. Deficiency of soil P is one of the most important chemical factors restricting plant growth in soils. The overfertilization of P leads to pollution due to soil erosion and runoff water containing large amounts of soluble phosphorus. Some microorganisms are known to be involved in the solubilization of insoluble phosphate (Hong *et al*, 2006).

1.2.3 Potassium

Potassium (K) concentrations in most plants range from 1 to 4% by weight. Unlike the other primary nutrients, K forms no other compounds in the plant, but remains a lone ion. Potassium is also vital for animal and human nutrition, and thus healthy fruits, vegetables and grains must have adequate levels of K (Brian, 2007).

Potassium regulates the opening and closing of the stomata by a potassium ion pump. Since stomata are important in water regulation, potassium reduces water loss from the leaves and increases drought tolerance. Potassium deficiency may cause necrosis or interveinal chlorosis. K+ is highly mobile and can aid in balancing the anion charges within the plant. It also has high solubility in water and leaches out of soils that rocky or sandy that can result in potassium deficiency. It serves as an activator of enzymes used in photosynthesis and respiration. Potassium is used to build cellulose and aids in photosynthesis by the formation of a chlorophyll precursor. Potassium deficiency may result in higher risk of pathogens, wilting, chlorosis, brown spotting, and higher chances of damage from frost and heat. (William, 2009).

Potassium fertilizers: Potassium chloride [KCl], Potassium sulfate [K_2SO_4], Potassium nitrate [KNO_3], Potassium-magnesium sulfate [K_2SO_4. $2MgSO_4$] (Silva & Uchida, 2000).

1.3 Secondary Macronutrients

Sulfur (S), calcium (Ca), and magnesium (Mg) are considered secondary macronutrients because they are less commonly yield-limiting than the primary macronutrients (N, P, and K), yet are required by crops in relatively large amounts (Nathan *et al*, 2005).

1.3.1 Calcium

Calcium is one of the main secondary nutrients necessary for healthy plant growth. Important sources of calcium are various fertilizers such as a single and a triple superphosphate, a nitrophoska, a precipitate, a calcium nitrate, etc. The other way for enriching soils by calcium is liming. For this aim a lime, a dolomite, a magnesite and various calcium carbonate minerals are used (Paleckienė *et al,* 2006).

1.3.2 Magnesium (Mg)

Magnesium is an essential component of chlorophyll, so it is essential for photosynthesis. It also regulates the uptake of other essential elements; serves as a carrier of phosphorus compounds, facilitates translocation and metabolism of carbohydrates. It considered as highly mobile nutrient in plants; relatively immobile in soils. Magnesium is an activator and component of many plant enzymes required in growth process, and enhances production of oils and fats (Jay, 2006). Magnesium fertilizers include: Dolomite [$CaMg(CO_3)_2$], Magnesium sulfate, Epsom salts [$MgSO_4.7H_2O$] , Magnesium oxide [MgO] contains 55% Mg (Silva & Uchida, 2000).

1.3.3 Sulfur (S)

Integral component of amino acids, therefore essential to protein synthesis. It considered as essential component of oils in aromatic compounds (e.g., garlic and onion), production of chlorophyll, essential for nodule formation on legume roots, increases size and weight of grain crops, aids in seed production. Highly mobile nutrient in plants; mobile in soils (Jay, 2006).

1.4 The Micronutrients or Trace Minerals

Boron (Bo), affects water absorption by roots Translocation of sugars; chlorine (Cl), is an essential to some plant processes, acts in the enzyme systems; manganese (Mn), is essential in plant metabolism, nitrogen transformation); iron (Fe), helps in carrying electrons to mix oxygen with other elements; zinc (Zn), is important in plants metabolism, helps form growth hormones, and reproduction; copper (Cu), helps in the use of iron, and helps respiration; molybdenum (Mo), improve plant development, reproduction, and selenium (Se) (Lee, 2008).

The macronutrients are consumed in larger quantities and are present in plant tissue in quantities from 0.2% to 4.0% (on a dry matter weight basis). Micronutrients are consumed in smaller quantities and are present in plant tissue in quantities measured in parts per million (ppm), ranging from 5 to 200 ppm, or less than 0.02% dry weight (wikipedia.org/wiki/ Fertilizer).

1.5 Types of Fertilizers

Among the materials used in agriculture, fertilizer is the most widely used. Based on the production process, it can be roughly categorized into three types: chemical, organic and biofertilizer (Jen-Hshuan, 2006).

1.5.1 Chemical Fertilizer (Synthetic Fertilizer)

Fertilizer**s** play an important role in increasing crop production. The main macronutrients present in inorganic fertilizers are nitrogen, phosphorus, and potassium which influence vegetative and reproductive phase of plant growth (Patil, 2010). Chemical Fertilizer is often synthesized using the Haber-Bosch process, which produces ammonia as the end product. This ammonia is used as a feed stock for other nitrogen fertilizers, such as anhydrous ammonium nitrate and urea. These concentrated products may be diluted with water to form a concentrated liquid fertilizer. Ammonia can be combined with rock phosphate and potassium fertilizer to produce compound fertilizer (wikipedia.org/wiki/Fertilizer).

1.5.1.1 The Advantages of Using Chemical Fertilizers

Nutrients are soluble and available to the plants, therefore the effect is direct and fast, The price is lower and more competitive than organic fertilizer, which makes it more acceptable and often applied by users, They are quite high in nutrient content; only relatively small amounts are required for crop growth (Jen-Hshuan, 2006).

1.5.1.2 Disadvantages of Chemical Fertilizers

The use of chemical fertilizers alone has not been helpful under intensive agriculture because it aggravates soil degradation. The degradation is brought about by loss of organic matter which consequently results in soil acidity, nutrient imbalance and low crop yields**,** Due to its high solubility, up to 70% of inorganic fertilizer can be lost through leaching, denitrification and erosion and reducing their effectiveness. (Ayoola, and Makinde, 2007**,** Alimi *et al*, 2007). Overapplication can result in negative effects such as leaching, pollution of water resources, destruction of microorganisms and friendly insects, crop susceptibility to disease attack, acidification or alkalization of the soil or reduction in soil fertility, thus causing irreparable damage to the overall system (Jen-Hshuan, 2006).

1.5.2 Organic Fertilizer

Organic fertilizer refers to materials used as fertilizer that occur regularly in nature, usually as a byproduct or end product of a naturally occurring process. Like any fertilizer, organic fertilizers typically provide the three major macronutrients required by plants: nitrogen, phosphorus, and potassium**.** Organic fertilizers include naturally-occurring organic materials, (e.g. manure, worm castings, compost, seaweed), or naturally occurring mineral deposits (wikipedia.org/wiki/Fertilizer). Organic fertilizers such as manure have been used in agriculture for thousands of years. Only within the past 100 years have fertilizers containing essential micro and macronutrients been synthesized in the laboratory (Thomas *et al,* 1990). In addition to increasing yield and fertilizing plants directly, organic fertilizers can improve the biodiversity (soil life) and long-term productivity of soil, and may prove a large depository for excess carbon dioxide. Organic nutrients increase the abundance of soil organisms by providing organic matter and micronutrients for organisms such as fungal mycorrhiza, (which aid plants in absorbing nutrients), and can drastically reduce external inputs of pesticides, energy and fertilizer, at the cost of decreased yield (wikipedia.org/wiki/Fertilizer).

1.5.2.1 Types of Organic Fertilizers

1- Animal manures

Animal manures are probably the most commonly available organic material used for their fertilizer value. Animal manure is essentially a complete fertilizer (Savoy, 1999).

2- Sewage sludge

It is a recycled product of municipal sewage treatment plants. Forms commonly available are activated, composted and lime-stabilized (Savoy, 1999).

3- Plant substances

They are often rich in specific nutrients, such as nitrogen.

4**- Composts**

Although making compost from a variety of raw materials is possible, the finished products are remarkably similar in their final concentrations of nitrogen, phosphorus, and potassium.

1.5.2.2 Advantages of Organic Fertilizers

Organic fertilizers are better sources of nutrient in balanced amounts than inorganic fertilizers where soil is deficient in both macro and micronutrients. Organic based fertilizer use is beneficial because it supplies micronutrients, and organic components that increase soil moisture retention and reduce leaching of nutrients. Nutrients in organic fertilizer are released from by soil microbes at almost the same time and speed as required by plant needs. The slow release of nutrients makes it possible for farmers to apply a season's worth of plant food in one application with less chance of loss to runoff. Organic fertilizers can be used on acid tolerant and those better suited to neutral or alkaline conditions (Alimi *et al*, 2007).

1.5.2.3 Disadvantages of Organic Fertilizers

Hard to get, Not sterile, Low nutrient content, Generally costs significantly more than synthetic fertilizer, Organic certification requires documentation and regular inspections, Organic fertilizers still release nutrients into their surroundings; these nutrients can find their way into local streams, rivers, and estuaries just as nutrients from synthetic sources do (Thomas *et al*, 1990).

1.6 Biofertilizer

Biofertilizers are commonly called microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form through biological processes **(**Chandrasekar, *et al*, 2005; Selvakumar, 2009). Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Soil

microorganisms play an important role in soil processes that determine plant productivity. There is a continuum of bacterial presence in soil, rhizosphere, rhizoplane, and internal the plant tissues. Bacteria living in the soil are called free-living as they do not depend on root exudates for their survival. Some bacteria support plant growth indirectly, by improving growth restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens (Tilak *et al*, 2005). The interactions among the rhizosphere, the roots of higher plants and the soil borne microorganisms have a significant role in plant growth and development. The organic compounds, released by roots and bacteria, play an important role in the uptake of mineral nutrient. The hormones produced by the rhizosphere bacteria have direct effects on higher plants. The density of PGPB (*Plant Growth Promoting Bacteria*) depends on the soil status and so the human activities (Marianna *et al,* 2005). Biofertilizers can add 20-200kg N ha^{-1} (by fixation), liberate growth-promoting substances and increase crop yield by 10-50%. They are cheaper, pollution free, based on renewable energy sources and also improve soil tilth (Saeed *et al*, 2004).

The use of biofertilizers effectively enrich the soil and cost less than chemical fertilizers, which harm the environment and deplete non-renewable energy sources. Biofertilizers have definite advantage over chemical fertilizers. Chemical fertilizers supply over nitrogen whereas biofertilizers provide in addition to nitrogen certain growth promoting substances like hormones, vitamins , amino acids, etc., crops have to be provided with chemical fertilizers repeatedly to replenish the loss of nitrogen utilized for crop growth. On the other hand biofertilizers supply the nitrogen continuously throughout the entire period of crop growth in the field under favorable conditions. Continuous use of chemical fertilizers adversely affect the soil structure whereas biofertilizers when applied to soil improve the soil structure. The effects of chemical fertilizers are that they are toxic at higher doses.

Biofertilizers, however, have no toxic effects. Biofertilizers are commonly called as microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes. For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility

status and for enhancement of crop production by their biological activity in the rhizosphere (Contra costa, 2003, Patil, 2010). Chemical fertilizers are expensive, they disturb the equilibrium of agro-ecosystems and cause pollution to the environment. These problems may be avoided by the use of biofertilizers (Al-Khiat, 2006). The utilization of microbial products has several advantages over conventional chemicals for agricultural purposes: (1) microbial products are considered safer than many of the chemicals now in use; (2) neither toxic substances nor microbes themselves will be accumulated in the food chain; (3) self-replication of microbes circumvents the need for repeated application; (4) target organisms seldom develop resistance as is the case when chemical agents are used to eliminate the pests harmful to plant growth; and (5) properly developed biocontrol agents are not considered harmful to ecological processes or the environment (Wua *et al*, 2004).

1.7 Nitrogen Fixing Bacteria

Following photosynthesis, nitrogen fixation is the second most important process in crop production. Photosynthesis captures sunlight and produces energy, and nitrogen fixation uses nitrogen gas to form ammonium. Nitrogen fixation can provide for free up to 300-400kg N/ha/yr (Adam, 2005).

The atmosphere comprises of ~78% nitrogen as an inert gas, N_2, which is unavailable to plants. Above every hectare of ground there are ~80000 tones of this unavailable nitrogen. In order to be converted to available form it needs to be fixed through either the industrial process (Haber Bosh Process) or through biological nitrogen fixation (BNF). Without these nitrogen-fixers, life on this planet would probably disappear within a relatively short period of time (Benson, 2001; Crispina *et al*, 2002).

Biological nitrogen fixation refers to the process of micro-organisms fixing atmospheric nitrogen, mostly within subsoil plant nodules, and making it available for assimilation by plants, involves the conversion of nitrogen to ammonia by microorganisms using a complex enzyme system identified as nitrogenase . Other plants benefit from nitrogen-fixing bacteria when the bacteria die and release nitrogen to the environment or when the bacteria live in close association with the plant (Hannington,1997, Lindemann, 2008).

This process of biological nitrogen fixation (BNF) accounts for 65% of the nitrogen currently utilized in agriculture, which eighty percent comes from symbiotic associations and the rest from free-living or associative systems. These include: a) Symbiotic nitrogen fixing (N_2-fixing) forms, viz. *Rhizobium*, the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees, and b) Non-symbiotic (free-living, associative or endophytic) N_2-fixing forms such as *cyanobacteria*, *Azospirillum, Azotobacter, Acetobacter diazotrophicus , Azoarcus,* etc (Tilak *et al*, 2005; Rifat *et al*, 2010).

1.7.1 Benefits of Using BNF

1. Economics: BNF reduces costs of biofertilizers production.

2. Environment: The use of inoculants as alternatives to N fertilizer avoids problems of contamination of water resources from leaching and runoff of excess fertilizer.

3. Efficiency: Legume inoculants do not require high levels of energy for their production or distribution. Application on the seed is simple compared to spreading fertilizer on the field.

4. Better yields: Inoculants increase legume crop yields in many areas. BNF often improves the quality of dietary protein of legume seed even when yield increases are not detected.

5. Increased soil fertility: Through practices such as green manuring, crop rotations, and alley cropping, N fixing legumes can increase soil fertility, permeability, and organic matter to benefit non-legume crops.

6. Sustainability: Using BNF is part of the wise management of agricultural systems. The economic, environmental, and agronomic advantages of BNF make it a cornerstone of sustainable agricultural systems (Silva & Uchida, 2000).

1.7.2 Symbiotic Nitrogen Fixers

Symbiotic nitrogen fixation provides 80% of the biologically fixed nitrogen on land. Nitrogen fixing bacteria are very selective in choosing roots of particular legumes species to infect, invade and form root nodules (Chandrasekar *et al,* 2005). Two groups of nitrogen fixing bacteria, i.e. *Rhizobia* and *Frankia* have been studied extensively. *Frankia* forms root nodules on more than 280 species of woody plants from 8 different families (Tilak *et al*, 2005). There are multiple advantages to this kind

of symbiosis: Plants can provide nitrogen themselves, thus considerably increasing their protein content, it may provide nitrogen to associated crops of different plant species, it may leave nitrogen in the soil available for other crops(Jen-Hshuan, 2006).

The efficiency in the use of the fixed nitrogen by the plant is almost 100% as compared to only 50-60% using nitrogen fertilizers. The amount of symbiotically fixing nitrogen considerably varies, depending mainly on the specie of leguminous and the effectiveness of the *Rhizobium*, and climate conditions, cultivation management and, eventually of the cattle management. The values of N may fluctuate between 50 and 800 $kg.ha^{-1}.year^{-1}$. With these nitrogen contributions large quantities of nitrogen fertilizer could be substituted (Urzúa, 2005). The establishment and maintenance of an effective symbiosis depends on several factors of which a favorable environment, that will allow maximum N2 fixation, is extremely important. Several environmental factors such as soil pH, soil fertility, temperature extremes impose limitations on the symbiotic association between the host plant and microsymbiont (Neeraj *et al,* 2009).

1.7.2.1 Rhizobia

Rhizobia (species of *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, and *Sinorhizobium*) form intimate symbiotic relationships with legumes by responding chemotactically to flavonoid molecules released as signals by the legume host (Viviene & Felix, 2004). Even though people observed bump on legume roots as early as the 17th century. It took a German scientist, to recognize that the legume root nodules themselves were responsible for the conversion of atmospheric nitrogen to ammonia (1888). The organisms inside the nodule were thought by some to be vibrio-like or bacteria-like organisms, but others were of the opinion that they were fungi. The microorganisms were first isolated and cultured by Martinus Beijerinck (1888) from nodules of a number of different legume species (Ann, 2009).

Root infection by rhizobia is a multistep process that is initiated by preinfection events in the rhizosphere. Rhizobia respond by positive chemotaxis to plant root exudates and move toward localized sites on the legume roots. For many rhizobia, primary target sites for infection are young growing root hairs, but there are no exclusive loci for rhizobial attachment (Pieternel and Jos, 1995).

Rhizobium with non-legumes could act as phosphate solubilizer, hormone producer and to some extent as N-fixer. Inoculation with *Rhizobium* can consequently led to improved soil fertility and can reduce the production cost of next crop through reduced input in the form of nitrogen fertilizers, which in turn also minimize the health hazard effects (Noshin and sumera, 2008).

1.7.3 Non-Symbiotic and Associated Nitrogen Fixers

Non-symbiotic nitrogen fixation is known to be of great agronomic significance. The main limitation to non-symbiotic nitrogen fixation is the availability of carbon and energy source for the energy intensive nitrogen fixation process. This limitation can be compensated by moving closer to or inside the plants. Some important nonsymbiotic nitrogen-fixing bacteria include: *Achromobacter, Acetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Azomonas, Bacillus, Beijerinckia, Clostridium, Corynebacterium, Derxia, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas, Rhodospirillum, Rhodopseudomonas* and *Xanthobacter* (Tilak *et al*, 2005).

1.7.3.1 Azospirillum

Azospirillum plant interactions have been extensively studied since 1970s. The beneficial effect of *Azospirillum* may derive both from its nitrogen fixation and stimulating effect on root development (Wua, *et al*, 2004, Noshin & sumera, 2008). Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size and root length of cereals (Wua, *et al*, 2004). *Plant growth-promoting bacteria (PGPB)* of the genus *Azospirillum* are widely distributed in the rhizosphere of tropical and subtropical grasses (Gül, 2003).

The mechanisms by which *Azospirillum* spp. can exert a positive effect on plant growth is probably composed of multiple effects including synthesis of phyto-hormones, N_2-fixation, nitrate reductase activity and enhancing minerals uptake (El-Komy, 2004). *Azospirillum*–plant association is accompanied by biochemical changes in roots, which in turn; promote plant growth and tolerance to low soil moisture. The bacteria stimulate plant-growth even in the presence of several stresses such as drought (Noshin, *et al*, 2008).

1.7.3.2 Azotobacter

Azotobacter represents the main group of heterotrophic free living nitrogen-fixing bacteria. They are Gram negative, large ovoid pleomorphic cells of 1.5-2.0 µm or more in diameter ranging from rods to coccoid cells. They occur singly, in paired or irregular clumps and sometime in chains of varying length. They do not produce endospores but form cysts. They are motile by peritrichous flagella or non motile. *Azotobacter* spp. are most specifically noted for their nitrogen fixing ability but they have also been noted for their ability to produce different growth hormones (IAA and other auxins, such as gibberllins and cytokinins), vitamins and siderophores. *Azotobacter* is capable of converting nitrogen to ammonia, which in turn is taken up by the plants (Kamil, *et al*, 2008). *Azotobacter* sp. can also produce antifungal compounds to fight against many plant pathogens. (Jen-Hshuan, 2006).

1.7.4 Phosphate Solubilizing Microorganisms (PSMS)

Phosphate solubilizing bacteria (PSB) are used as biofertilizer since 1950's. These microorganisms secrete different types of organic acids e.g., carboxylic acid thus lowering the pH in the rhizosphere and consequently dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$ in calcareous soils. Efficiency of P fertilizer throughout the world is around 10 - 25 %, and concentration of bioavailable P in soil is very low reaching the level of 1.0 mg kg^{-1} soil. Among the whole microbial population in soil, PSB constitute 1 to 50 %, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5 % in P solubilization potential (Aftab and Asghari, 2008).

This group covers bacteria, fungi and some actinomycetes. These organisms solubilize the unavailable forms of inorganic-P like tricalcium, iron, aluminum and rock phosphates into soluble forms by release of a variety of organic acids like succinic, citric, malic, fumaric, glyoxalic and gluconic acids (Venkateswarlu *et al*, 2007). PSMs include different groups of microorganisms, which not only assimilate phosphorus from insoluble forms of phosphates, but they also cause a large portion of soluble phosphates to be released in quantities in excess of their requirements. Species of *Aspergillus* and *Penicillium* are among fungal isolates identified to have phosphate solubilizing capabilities. Among the bacterial genera with this capability are *Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Serratia, Enterobacter, Acinetobacter, Flavo-bacterium* and *Erwinia* (Richa, 2003). It

is reported that PSB culture increased yield up to 200-500 kg/ha and thus 30 to 50kg of superphosphate can be saved (Jen-Hshuan, 2006).

1.7.5 Potassium Solubilizing Bacteria

Potassium solubilizing bacteria (KSB) such as *Bacillus mucilagenosus* and *Bacillus edaphicus* are example of microorganisms that used in biofertilizer. KSB are able to solubilize potassium rock through production and secretion of organic acids. KSB is a heterotrophic bacterium which is obtaining all their energy and cellular carbon from preexisting organic material. Besides, KSB are aerobic bacteria which play an important role in maintaining soil structure by their contribution in the formation and stabilization of water-stable soil aggregates. In addition, this gram positive bacterium can produce substance that stimulate plant growth or inhibit root pathogens. (Bin Zakaria, 2009).

1.7.6 Plant Growth Promoting Rhizobacteria (PGPR)

Plant Growth Promoting Rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978) (Bin Zakaria, 2009). Are a group of bacteria that actively colonize plant roots and increase plant growth and yield. The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to include: the ability to produce phytohormons, asymbiotic N2 fixation, against phytopathogenic micro-organisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and also solubilization of mineral phosphates and other nutrients (Gholami, et al, 2009). Enhanced supply of other plant nutrients (P mobilization, S oxidation, Fe chelation), phytochrome production leading to increases in root surface area (IAA, cytokinin, gibberllin) (Heike, 2007).

Production of biologically active substances or plant growth regulators (PGRs), which is one of the major mechanisms through which PGPR influence the plant growth and development (Javed *et al*, 2009). Some PGPR may promote plant growth indirectly by affecting symbiotic N_2 fixation, nodulation or nodule occupancy. However, role of cyanide production is contradictory as it may be associated with deleterious as well as beneficial rhizobacteria. The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plant. The environmental factors include climate, weather conditions, soil

characteristics or the composition or activity of the indigenous microbial flora of the soil (Joseph, *et al*, 2007).

There are several types of rhizobacteria and the type is depending on the nutrients provided into the soil systems and mechanism used. Nowadays, biofertilizer are able to increase plants nutrients uptake by introducing nitrogen fixing bacteria associated with roots (*Azospirillium*) for nitrogen uptake, iron uptake from siderophore producing bacteria (*Pseudomonas*), sulfur uptake from sulfuroxidizing bacteria (*Thiobacillus*), phosphorus uptake from phosphate-mineral solubilizing bacteria (*Bacillus, Pseudomonas*) and potassium uptake from potassium solubilizing bacteria, KSB (*Bacillus*). These are the several types of PGPR that usually used in the biofertilizer and introduce into the soil and their mechanism take place at the rhizosphere (Bin Zakaria, 2009).

1.7.7 Vesicular Arbuscular Mycorrhizae (VAM)

The majority of plants growing under natural conditions are associated with mycorrhizae. Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The extrametrical fungal hyphae can extend several centimeters into the soil and absorb large amounts of nutrients for the host root (Wua *et al,* 2004). Mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots (Contra costa, 2003). Mycorrhiza is a mutualistic association between fungi and higher plants. Different types of mycorrhizae occur, distinguished by their morphology and to a certain extent, in their physiology. These include ectomycorrhizae (EM) and endomycorrhizae (AM) (Turk, *et al,* 2006).

While both types penetrate the plant roots, ectomycorrhizae spread their hyphae between root cells, while endomycorrhizae hyphae penetrate root cells (Contra costa, 2003). Symbiotic association of plant roots with VA-fungi often result in enhanced growth because of increased acquisition of phosphorus (P) and other low mobile mineral nutrients. VA-fungi are known to be effective in increasing nutrient uptake, particularly phosphorus and biomass accumulation of many crops in low phosphorus soil (Turk *et al*, 2006). Mycorrhizae also benefit plants indirectly by enhancing the structure of the soil. AM hyphae excrete gluey, sugar-based compounds called

Glomalin, which helps to bind soil particles, and make stable soil aggregates. This gives the soil structure, and improves air and water infiltration, as well as enhancing carbon and nutrient storage (Contra costa, 2003).

1.7.8 Blue Green Algae

Cyanobacteria play an important role in maintenance and build-up of soil fertility, and yield as a natural biofertilizer. The acts of these algae include: (1) Increase in soil pores with having filamentous structure and production of adhesive substances. (2) Excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, amino acids. (3) Increase in water-holding capacity through their jelly structure. (4) Increase in soil biomass after their death and decomposition. (5) Decrease in soil salinity. (6) Preventing weeds growth. (7) Increase in soil phosphate by excretion of organic acids (Saadatnia, 2009). Most of the *Cyanobacteria* can produce exo-polysaccharides. *Cyanobacteria* are structurally diverse assemblages of aerobic gram-negative eubacteria (Prokaryotes) characterized by their ability to form oxygenic photosynthesis. They reduce molecular atmospheric nitrogen to ammonium which can then be utilized for amino acid and protein biosyntheis (Padhi and Swain, 1996, Al-Khiat, 2006).

The species of cyanobacteria which are known to fix atmospheric nitrogen are classified into three groups (1) Heterocystous-aerobic forms, (2) Aerobic unicellular forms and (3) Non-heterocystous, filamentous, microaerophilic forms. *Cyanobacteria* that dominate a wide range of diverse environments are characterized by their tolerance to high temperatures, desiccation, pH, salinity, light intensity and nutrients. *Anabaena sp*. and *Nostoc sp*. are the most common nitrogen fixing organisms in rice fields, mostly occurring as free floating water blooms forming a microbiological mat. Similarly, more than 100 strains of heterocystous cyanobacteria belonging to the genera *Anabaena, Nostoc, Nodularia, Cylindrospermum, Scytonema, Calothrix, Anabaenopsis, Mastigocladus, Fischerella, Tolypothrix, Aulosira, Stigonema, Hapalosiphon, Chlorogloeopsis, Cauptylonema, Gloeotrichia, Nostochopsis, Rivularia, Westiellopsis, Westiella, Schytonematopsis, Wollea* and *Chlorogloea* have been found to be efficient as N2 fixers (Al-Khiat, 2006).

1.7.9 Azolla

Azolla is a free floating fresh water fern belonging to the family Azollaceae and order Pteridophyta. There are six species of Azolla. It is commonly found in tropics and sub-tropics. It grows naturally in stagnant water of drains, canals, ponds, rivers. *Azolla* sp. is unique among floating macrophytes, because it can grow in waters devoid of combined nitrogen, due to the symbiosis with a N2 fixing cyanobacterium, *Anabaena azollae*, that lives in the dorsal lobe cavity of its leaf. Azolla is rich in protein, total protein is 25-30%. Other constituents in Azolla are minerals, chlorophyll, carotinoids, amino acids, vitamins etc. It is also a potential source of nitrogen (Lourdes *et al*, 1999, Biplob *et al*, 2002).

1.8 Significance of the Study

Gaza Strip is an agricultural land with a shortage of water resources, and a very densely populated area. Farmers use chemical fertilizers to increase production to meet their needs, but the excessive use of fertilizers leads to contamination of soil and groundwater and reduce soil fertility. As the purchase of chemical fertilizers are difficult and expensive as a result of the blockade, its known that the excessive use of chemical fertilizers have generated several environmental problems including the greenhouse effect, ozone layer depletion, acidification of water, and pollution of water resources, destruction of micro-organisms, acidification or alkalization of the soil or reduction in soil fertility. So biofertilizers can replace partially chemical fertilizers. Hence there is a need to search for alternative strategies to improve soil health without causing damage to environment as well as soil. Therefore biofertilizers are gaining the importance as they are ecofriendly, non hazardous and nontoxic products (Sharma, *et al*, 2007).

1.9 The Aim of the Study

1.9.1 General Objectives

Study the effects of *Azotobacter chroococcum* as nitrogen-biofertilizers on growth and yield of *Cucumis sativus* (Cucumber).

1.9.2 Specific Objectives

1. Isolation, identification, and cultivation of local strain of *Azotobacter chroococcum*.
2. Evaluation the effectiveness of *A.chroococcum* as biofertilizer in growth and yield of *Cucumis sativus* compared with the control samples and other fertilizers.

Chapter 2

Literature Review

2.1 Azotobacteraceae

Two genera of bacteria in family Azotobacteraceae that fix nitrogen as free-living organisms under aerobic conditions: *Azotobacter* and *Azomonas.* The basic difference between these two genera is that *Azotobacte*r produces drought-resistant cysts and *Azomonas* does not. Aside from the presence or absence of cysts, these two genera are very similar. Both are large gram-negative motile rods that may be ovoid or coccoidal in shape (pleomorphic). Catalase is produced by both genera. There are six species of *Azotobacter* and three species of *Azomonas* (Jan, 2006).

Although some *rhizobia* may fix nitrogen nonsymbiotically, unlike *Azotobacter*, they can only do so under reduced oxygen tension. Furthermore, their cells are generally smaller than Azotobacter cells (*A. paspali* excepted). Moreover *rhizobia* need a more complex medium (supplemented with growth substances, etc.) for growth .Other nonsymbiotic nitrogen-fixing organisms have a different cell morphology and widely different physiological and nutritional requirements depending on the taxonomic group of the prokaryote class to which they belong (Jan, 2006). Differentiation of the six species of the genus *Azotobacter* and three species of *Azomonas* is based primarily on the presence or absence of motility, the type of water-soluble pigment produced, and carbon source utilization. Four species of *Azotobacter* and all three species of *Azomonas* are motile. Pigmentation these organisms produce both water-soluble and water-insoluble pigments (Benson, 2001).

2.1.1 Azotobacter

The first species of the genus *Azotobacter*, named *Azotobacter chroococcum*, was isolated from the soil in Holland in 1901. These nitrogen-fixing bacteria are important for ecology and agriculture (Mrkovac & Milic, 2001). Free-living, aerobic N_2 fixing bacteria of the genus *Azotobacter* were discovered at the turn of the century (Beijerinck, 1901) and their N_2 Fixing associations with plants were then soon investigated to improve the productivity of non-leguminous crops (Hong *et al*, 2006). Azotobacter is able to fix at least 10 mg N per gram of carbohydrate (Tejera, *et al*, 2004).

Although the free-living Azotobacteraceae are beneficial nitrogen-fixers, their contribution to nitrogen enrichment of the soil is limited due to the fact that they would rather utilize NH_3 in soil than fix nitrogen. In other words, if ammonia is present in the soil, nitrogen fixation by these organisms is suppressed (Benson, 2001). Among the free-living nitrogen-fixing bacteria, those from genus *Azotobacter* have an important role, being broadly dispersed in many environments such as soil, water and sediments (Mirjana *et al*, 2006). *Azotobacter sp*, are free-living aerobic bacteria dominantly found in soils, present in alkaline and neutral soils. They are non-symbiotic heterotrophic bacteria capable of fixing an average 20kg N/ha/year. Besides, it also produces growth promoting substances and are shown to be antagonistic to pathogens. *Azotobacter sp*. are found in the soil and rhizosphere of many plants and their population ranges from negligible to 10^4 g^{-1} of soil depending upon the physico-chemical and microbiological (microbial interactions) properties (Ridvan, 2009).

In soils, *Azotobacter sp*. populations are affected by soil physico-chemical (e.g. organic matter, ph, temperature, soil depth, soil moisture) and microbiological (e.g. microbial interactions) properties (Ridvan, 2009). The genus Azotobacter includes 6 species, with *A. chroococcum* most commonly inhabiting various soils all over the world. The occurrence of other *Azotobacter* species is much more restricted in nature, e.g. *A. paspali* can be found only in the rhizosphere of a grass. Soil populations of *Azotobacter sp*. rarely exceed several thousand cells per gram of neutral or alkaline soils, and in acid (pH < 6.0) soils these bacteria are generally absent or occur in very low numbers (Martyniuk and Martyniuk, 2002). *Azotobacter sp.* is gram negative bacteria, polymorphic i.e. they are of different sizes and shapes.

Old population of bacteria includes encapsulated forms and have enhanced resistant to heat, desication and adverse conditions. The cyst germinates under favorable conditions to give vegetative cells. They also produce polysaccha-rides. These are free living bacteria which grow well on a nitrogen free medium. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis (Khanafari *et al,* 2006). The genus *Azotobacter* comprises large, gram-negative, primarily found in neutral to alkaline soils, obligately aerobic rods capable of fixing N_2 nonsymbiotically. Azotobacter is also of interest because it has the highest respiratory rate of any living

organism. In addition to its ecological and physiological importance, *Azotobacter* is of interest because of its ability to form an unusual resting structure called a cyst. *Azotobacter* cells are rather large for bacteria, many isolates being almost the size of yeast, with diameter of 2-4 µm or more (Gül, 2003).

Besides, nitrogen fixation, *Azotobacter* also produces, thiamin, riboflavin, indole acetic acid and gibberellins. When *Azotobacte*r is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substances produced by *Azotobacter*. The exact mode of action by which *azotobacteria* enhances plant growth is not yet fully understood. Three possible mechanisms have been proposed: N_2 fixation; delivering combined nitrogen to the plant; the production of phytohormone-like substances that alter plant growth and morphology, and bacterial nitrate reduction, which increases nitrogen accumulation in inoculated plants (Mrkovac & Milic, 2001).

2.1.1.1 Effect of External Environmental Factors on the Growth of the Genus *Azotobacter*

1. PH Effect

The presence of *A. chroococcum* in soil or water is strongly governed by the pH value of these substrates. In an environment below pH 6.0, *Azotobacter* is rare or absent. The soils above pH 7.5 contained A. chroococcum varying in numbers between 10^2 and 10^4 per gram of soil. In nitrogen-free nutrient media, the lower pH limit for growth of *A. chroococcum* strains in pure culture is between pH 5.5 and 6.0 (Jan, 2006).

2. Temperature

In relation to temperature, *Azotobacter* is a typical mesophilic organism. Most investigators regard 25-30ºC as the optimum temperature for *Azotobacter* . The minimum temperature of growth of *Azotobacter* evidently lies a little above 0ºC. Vegetative *Azotobacter* cells cannot tolerate high temperatures, and if kept at 45-48ºC they degenerate (Gül, 2003).

3. Aeration

Owing to the fact that *Azotobacter* is an aerobe, this organism requires oxygen. As many investigators have noted, aeration encourages the propagation of *Azotobacter*. Effect of different oxygen tensions on the biomass formation of *A. vinelandii* was studied and shown that biomass formation was optimum at PO_2 2-3% (air saturation)

and decreased with increasing PO_2. In another study, both increasing dissolved oxygen tension and increasing agitation speed increased cell concentration of *Azotobacter* when grown diazotrophically. The initiation of growth of nitrogen-fixing Azotobacter species was prevented by efficient aeration but proceeded normally with gentle aeration (Gül, 2003).

4. Inorganic Salts

Azotobacter needs some basic nutrient to proliferate in nitrogen-free medium. Beside the carbon source, it needs several salts to fix nitrogen so to propagate. Iron and molybdenum are the co-factors of the nitrogenase enzyme, responsible for the nitrogen fixation, so essential for growth. The propagation of *Azotobacter* is largely dependent on the presence of phosphorous and potassium compounds in the medium. Calcium and magnesium play an important role in the metabolism of *Azotobacter*. Although manganese is evidently not an essential element for nitrogen fixation, its favorable action was reported with the highest requirement of *A. chrooccocum* at the 20-30 ppm in the medium. According to the information about the action of copper on *Azotobacter* is toxic even in very low concentrations (Gül, 2003).

5. Nitrogen

Although *Azotobacters* in general are nitrogen fixers, addition of nitrogen in the medium decreases the lag phase and generation time and thus fermentation time. When nitrogen is supplied in the $NaNO_3$ form, up to 0.5 g/L concentration, there was an increase in growth, but further increases in concentration did not altered the growth pattern. The best results are obtained with NH_4Cl form at 0.1 g/L (Gül, 2003).

2.1.1.2 Production of Growth Substances and their Effects on the Plant

Growth substances, or plant hormones, are natural substances that are produced by microorganisms and plants alike. they have stimulatory or inhibitory effects on certain physiological-biochemical processes in plants and microorganisms. *Azotobacteria* produced indol-3-acetic acid (IAA) when tryptophan was added to the medium, on the other hand, found only small amounts of IAA in old cultures of *Azotobacteria* to which no tryptophan was added. three gibberelin-like substances were detected in an *Azotobacter chrococcum* strain. The amounts found in the 14-dayold cultures ranged between 0.01 and 0.1 µg gaz equivalent/ml. Bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and GA-like substances. These hormonal substances,

which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants (Mrkovac & Milic, 2001).

2.1.2 *Azotobacter chroococcum*

Table 2.1 Taxonomy of *Azotobacter chroococcum*

Bacteria	Domain
Proteobacteria	Phylum
Gammaproteobacteria	Class
Pseudomonadales	Order
Pseudomonadaceae/Azotobacteraceae	Family
Azotobacter	Genus
Azotobacter chrococcum	Species

Characteristic sings of *A. chroococcum* as follows; Size of cell 3.1 x 2.0 µm; Forms cyst; Motile, especially in young culture or if grown in ethanol; The colonies of *A.chroococcum* at free nitrogen media were slightly viscous, semi-transparent at first, later dark-brown. Utilizes starch; In some cases utilizes sodium benzoate; utilizes mannitol benzoate; utilizes rhamnose benzoate (Martinez *et al*, 1985, Gül, 2003).

Cells of *A. chroococcum* are pleomorphic, bluntly rod, oval or coccus-shaped. Mean dimensions are 3.0–7.0 µm long × 1.5–2.3µm wide. The cell shape changes dramatically in time or with changes in growth (medium) conditions. Cells are often in pairs show figure 2.1 . Young cells are motile by peritrichous flagella. Microcysts and capsular slime are formed. Colonies are moderately slimy, turning black or black-brown on aging, the pigment produced is not water-diffusible (Jan, 2006).

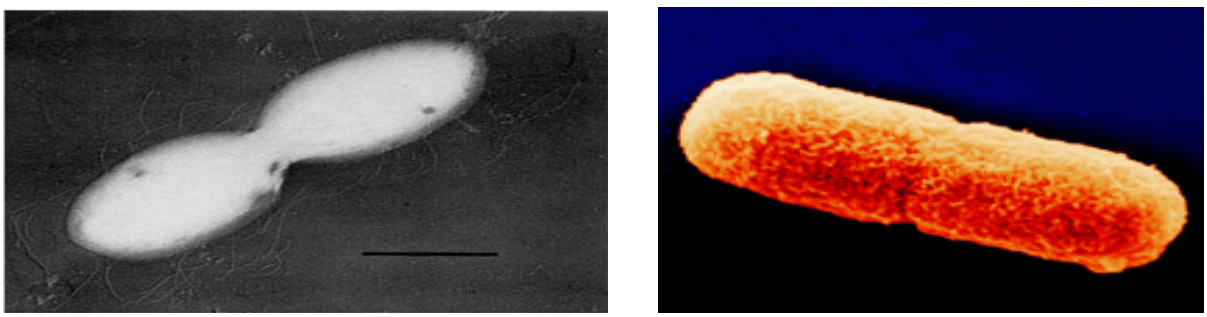

Figure 2.1*. Azotobacter chroococcum*. Two cells in a pair

Azotobacter chroococcum, a free-living diazotroph has also been reported to produce beneficial effects on crop yield through a variety of mechanisms including

biosynthesis of biologically active substances, stimulation of rhizospheric microbes, modification of nutrient uptake and ultimately boosting biological nitrogen fixation.

The presence of *A. chroococcum* in soil or water is strongly governed by the pH value of these substrates. In an environment below pH 6.0, Azotobacter is generally rare or totally absent. Soils above pH 7.5 contained Azotobacter (predominantly *A. chroococcum*) varying in numbers between 10^2 and 10^4 per gram of soil (Jan, 2006; Qureshi *et al*, 2009). Due to the role of *A. chroococcum* in nitrogen fixation, It is an important (PGPR) producing compounds needed for plant growth and to their potential biotechnological applications. *A. chroococcum* produces gibberelins, auxins, and cytokinins (Mrkovac and Milic, 2001).

2.1.3 The Cucumber (*Cucumis sativus L*.)

Table 2.2. Taxonomy of *Cucumis sativus L*.

Plantae	Kingdom
Magnoliophyta	Division
Magnoliopsida	Class
Cucurbitales	Order
Cucurbitaceae	Family
Cucumis	Genus
C. sativus	Species

Cucumber, *Cucumis sativus L*., is one of the most popular members of the cucurbitaceae family of Bengal. Cucumber has been known in history for over 5000 years. From India, cultivation migrated to Greece, Italy and China before arriving in Europe as early as the 9th century and records of cucumber cultivation appear in France in the 9th century, in England in the 14th century and in North America by the mid-16th century (Nahit, 2004). Cucumber (*Cucumis sativus* L.) is a tender annual vegetable vine crop, grown for its fresh fruit. It is used as salads or taken as fresh fruit desserts. In addition to its delicious taste and fairly good caloric value, it has high medicinal value for human beings. It is well known for natural diuretic and thus can serve as an active drug for secreting and promoting flow of urine. Due to high content of potassium (50-80 mg/100g), cucumber can highly be useful for both high and low blood pressures (Kashif *et al*, 2008).

The cucumber is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around ribbing with thin, spiraling tendrils. The plant has large leaves that form a canopy over the fruit. The fruit is roughly cylindrical, elongated, with tapered ends, and may be as large as 60cm long and 10cm in diameter. Cucumbers are mainly eaten in the unripe green form. The ripe yellow form normally becomes too bitter and sour. Cucumbers are usually over 90% water. Having an enclosed seed and developing from a flower, botanically speaking, cucumbers are classified as fruits. However, much like tomatoes and squash they are usually perceived, prepared and eaten as vegetables (http://en.wikipedia. org/wiki/-Cucumber).

Figure 2.2. Cucumber

Cucumber is a member of the Cucurbitaceae family, which comprises 90 genera and 750 species. Besides *Cucumis sativus L*. the genus *Cucumis* comprises about 30 different species which are distributed over two geographically separated areas. The first called as “African group” is spread over large parts of Africa and the Middle East to Pakistan and south Arabia and this group contains the larger portion of the species. The second called as “Asiatic group” can be found in the areas south and east of the Himalayas and *C. sativus L*. belongs to this group. Cucumber has been grown and bred for centuries and both as a vegetable crop and for medicinal purposes.

The origin and domestication of cucumber was probably not in the Middle East, however, nor in Africa as some have suggested, but rather in Asia. Cucumber is originally a monoecious plant species. In East Asian varieties, purely female, and in the Australian variety White Lemon, andromonoecious plants, i.e., plants with staminate and perfect (hermaphrodite) flowers have also been described. Thus, there are three different flower types in cucumber. These are pistillate, staminate and hermaphrodite flowers. According to the various distributions of these flower types on the plants, the different sex types result: monoecious, gynoecious, androecious, hermaphroditic and andromonoecious. Hermaphroditic plants have only herma-

phrodite flowers, gynoecious plants only pistillate and androecious plants only staminate flowers. Cucumber is the fourth important vegetable crop after tomato, cabbage and onion. It is cultivated in nearly all countries of temperature zones and growing best at temperatures above 20°C (Nahit, 2004). Cucumber is a semi-tropical vegetable crop, and grows best under the conditions of high light, humidity, moister, temperature and fertilizer. Its growth habit is indeterminate. The plants produce fruit continuously where diseases and insects are controlled. Cucumber is very sensitive to low temperatures, which may cause reductions in both growth and yield. Cucumber is very sensitive to N deficiency, which can alter the fruit shape, and is intolerant of salinity. Deficiencies of Mg and of B, Fe and Mn, can occur and demand direct application of these nutrients ((Nahit, 2004).

2.1.4 Inoculation of Biofertilizers

2.1.4.1 Seed Inoculation

Seed inoculation uses a specific strain of microbe that can grow in association with plant roots; soil conditions have to be favorable for the inoculants to perform well. Selected strains of N-fixing Rhizobium bacteria have proven to be effective as seed inoculants for legumes. The seed treatment can be done with any of two or more bacteria without antagonistic effect. In the case of seed treatment with *Rhizobium, Azotobacter, Azospirillum* along with PSB, first the seeds must be coated with *Rhizobium* or *Azotobacter* or *Azospirillum*. When each seed has a layer of the aforesaid bacteria then the PSB inoculant has to be treated on the outer layer of the seeds. This method will provide maximum numbers of population of each bacterium to generate better results (Jen-Hshuan, 2006).

2.1.4.2 Soil inoculation

In soil inoculation, microbes are added directly to the soil where they have to compete with microbes already living in the soil that are already adapted to local conditions and greatly outnumber the inoculums. Inoculants of mixed cultures of beneficial microorganisms have considerable potential for controlling the soil microbiological equilibrium and providing a more favorable environment for plant growth and protection. Therefore, adequate quality control and a high level of consistency in performance and benefits must be ensured. Although inoculations with PSBs have

not been very effective, joint inoculation of PSBs with mycorrhizae and N2-fixing bacteria have been successful (Jen-Hshuan, 2006).

2.1.5 Preview of Previous Studies

About 77 different microbial isolates (24 *Azotobacter*, 14 *Bacillus*, 9 *Pseudomonas*, 14 *Actinomycetes* and 16 *Fungi*), were isolated. Selected effective microorganism showed high compatibility when mixed together. *Azotobacter chroococcum* recorded the highest values of carbohydrates and microbial gum production. Wheat growth criteria (shoot length, root length, shoot fresh and dry weight, root fresh and dry weight, chlorophyll content, number of leaves), yield parameters, mineral content (NPK) of wheat in soil rhizosphere and in plant were measured and, increased by inoculation (Abd El-Ghany *et al*, 2010).

To evaluate the efficiency of *Azotobacter*, *Azospirillum* and their combination on plant growth and yield parameters of *Brassica juncea* cv. Varuna, *Azotobacter* and *Azospirillum* were applied separately and combination of both the bacteria in half doses. Application of both the bacteria recorded higher plant growth and yield in *Brassica juncea*. *Azospirillum* inoculation resulted in higher growth and yield parameters in comparison to *Azotobacter* inoculation. However, the combination of half dose of both the bacteria proved best in improving plant growth andyield in comparison to individual inoculation **(**Irfan *et al*, 2010).

A factorial experiment in the form of complete randomized block design with three replications has been used. Inoculation of *Azotobacter* (without and with inoculation by *Azotobacter chroococum*) and *Mycorrhiza* (without and with inoculation by *Glomus intraradices*) under different levels of nitrogen and phosphorus levels, on spring safflower have been studied. Seed inoculation at the planting date with *Azotobacter* and *Mycorrhiza* caused increasing grain yield about 6.13% in compare with control treatment. Conclusion: Seed yield and yield components of safflower have been affected significantly by the inoculation with *Azotobacter* and *Mycorrhiza* **(**Mirzakhani *et al*, 2009).

Field experiment was conducted to assess the co-inoculation potential of symbiotic i.e. *Mesorhizobium ciceri* and non-symbiotic diazotrophs i.e. *Azotobacter chroococcum* on the yield of chickpea. It was observed that inoculation with *M. ciceri*

or *A. chroococcum* produced significant increase in biomass and grain yield. Percent N and P content in chickpea plant were higher in the co-inoculated treatments than that of their respective controls. Similar trend was observed in grains except the rhizobial inoculation alone which produced higher N content than co-inoculation. Percent N and available P in soil were also higher in the inoculated treatments (Qureshi *et al*, 2009).

To evaluate the response of biofertilizer and inorganic fertilizer on germination and growth of tomato plant, nitrogen (N) was used as inorganic fertilizer and *Azotobacter* was used as biofertilizer. The conclusion was that *Azotobacter* as biofertilizer reported better than inorganic fertilizer in relation to seed germination and all plant growth parameters (Mahato *et al,* 2009).

The objective of greenhouse study was to evaluate the effects of chemical fertilizers (N and P) against two biofertilizers containing N-fixer bacteria (*Azotobacter chroococcum*) and P solubilizing bacteria (*Bacillus megaterium*) and ATP (adinosine tri-phosphate) on the growth parameters and quality of fatty acid fraction of *Matthiola incana*. The use of biofertilizer resulted in the highest biomass and seedling height. This greenhouse study also indicated that the biofertilizer application had similar effects when compared with chemical fertilizer treatments. (Rawia *et al*, 2009).

The present investigation was carried to study the effect of some bacterial inoculation with *Rhizobium leguminoarum* bv. *phaseoli* (ARC 301) (Rh) and two strains of *Azotobacter chroococcum* (AZ1) and *Bacillus megaterium* var phosphaticium (BM3) as a biofertilizers. The highest values were recorded with mixed inoculation treatment of Rh + AZ1 + BM3 in the presence of 25% from the recommended dose of chemical NPK fertilizers. The best interaction treatments regarding plant growth and chlorophyll leaf content was inoculation *cv. Paulista* with Rh + BM3 + 25% NPK (Gharib *et al*, 2009).

An experiment was conducted to determine the effect of biofertilizers on growth and yield of blackgram in field condition. The different inoculation (single and dual) of biofertilizers *Azotobacter, Azospirillum, Rhizobium,* phosphobacteria were incorporated into the top 15cm of the soil. The results revealed that addition the combination inoculation of *Rhizobium* + *phosphobacteria* significantly increased

growth and yield of blackgram compared with control (without biofertilizers) (Selvakumar *et al*, 2009).

A. chroococcum, belonging to the community of PGPR was used to study their effect on the growth of Bamboo (*Bambusa bamboo*) and Maize (*Zea mays*). It was found that *A. chroococcum* at concentration of 10^8 cfu ml^{-1} increased seed germination. It was also concluded that *Azotobacter* inoculants have a significant promoting effect on growth parameters like root, shoot length and dry mass of bamboo and maize seedlings in *invitro* and in pot experiments.. Therefore the present study suggest that *A. chroococcum* is beneficial for bamboo and maize plantation (Dhamangaonkar, 2009).

The yield parameters and cost economics of *Withania somnifera* were studied using dual inoculation of *A. chroococcum* and *Pseudomonas putida*. All quantitative plant traits increased significantly in response to organic manure. This response was enhanced further with bacterial inoculation + organic manure. The survival count of inoculated bacteria was highest 70 days after inoculation and declined thereafter. (Vivek *et al*, 2009).

Roots of young 'Golden Delicious' apple on M9 rootstock were inoculated with four strains of A. *chroococcum*. Therefore, a factorial arrangement included four strains of *A. chroococcum*, two levels of N-fertilizerand two levels of compost. Among the four strains, AFA_{146} was the most beneficial strain, as it increased leaf area, leaf potassium, magnesium, iron, manganese, zinc, and boron uptake and root nitrogen, phosphorus, potassium, manganese, and zinc. The combination of AFA_{146} strain, compost and N fertilizer increased leaf uptake of Ca, Mg, Fe, Mn, Zn, and B, and root uptake of P, K, Ca, Mg, Mn, and copper (Cu), and root dry weight. (Khosravi; *et al,* 2009).

In order to evaluation of the effect of *A. chroococum* on two varieties of wheat grown under field conditions, an experiment was carried out in Agricultural Research Station of Shahrood University of Technology during 2004-2006. Results showed that wheat yield was affected when cultivars inoculated. Inoculation resulted in improving post harvest seed germination and nitrogen content of the seed (Hamid, 2008).

An experiment was carried out to study the growth promotion of rice (*Oryza sativa* L.) due to dual inoculation of *A. chroococcum* and *Piriformospora indica* along with vermicompost. The effects on shoot length, root length, fresh shoot and root weight, dry shoot and root weight, and panicle number were investigated. Dual inoculated plants in presence of vermicompost gave better positive effects, in comparison to single inoculation of *A. chroococcum*, P.indica and vermicompost. This suggested that dual inoculation of *A. chroococcum* and P.indica had beneficiary response on growth of rice plant (Kamil *et al,* 2008).

Combined N-fixer (*Azospirillum brasiliensis, A. chroococcum*) and P solubilizer (*Bacillus megaterium*) bacteria with earthworms (*Glossoscolecidae, Pontoscolex corethrurus*); was set up to investigate the effects of biofertilizers and earthworms on maize and bean growth. Treatments that combined earthworms and biofertilizers promoted the highest growth of *P. vulgaris* (earthworms with *A. chroococcum*), the highest dry plant mass was enhanced by *Azospirillum brasiliensis for Z. mays*, and the highest yield production for *Z. mays* was enhanced with the presence of earthworms (earthworms with *A. chroococcum* and earthworms with *Bacillus megaterium*), 4-fold higher than control (Huerta *et al*, 2007).

Seeds of wheat (*Triticum Aestivum*) were inoculated with 11 bacterial strains of *A. chroococcum*, Research result showed that all *A. chroococcum* strains had positive effect on the yield and N concentrations of wheat (Ridvan, 2008).

Seeds of spring wheat were inoculated with some *A. chroococcum* strains. The selected strains had a significant effect on wheat growth and yield, including biological yield and seed quality under greenhouse conditions (Rajaee, *et al,* 2007).

Cucumber plants (*Cucumis sativus L. 'Passandra'* and *'Girola'*) were inoculated with two series of N-fixing bacteria (*A. chroococcum, Azospirillum brasilense*) and *Glomus mosseae* fungus. Inoculation with microorganisms did not affect P and total yield, but early yields were significantly increased in the case of inoculation with A. brasilense, alone or combined with *G. mosseae*, compared to the control. Inoculation with *A.*

chroococcum alone increased K concentrations in leaves, while the combined inoculation of *A. chroococcum* and *G. mosseae* increased N concentration in fruit tissues (Abdelaziz and Pokluda, 2007).
Research trials to test the effect of the inoculums of *Azotobacter* and *Azospirillum* on the yields of wheat in 2005-06 and 2006-07. In 2005-06, grain yield of inoculated irrigated wheat increased by 11%, while the yields of rainfed barley increased by 36% compared to the untreated control. In 2006-07, grain yields of inoculated rainfed wheat increased by 11% on average (Milani & Anthofer, 2007).

In field experiments during two successive seasons (2003-2004 and 2004-2005), a mixture of *A.* chroococcum*, Azospirillum liboferum,* and *Bacillus megatherium* applied with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth (plant height, number of branches, and herb fresh and dry weight per plant compared to chemical fertilizer treatments only. The tallest plants, the highest number of branches per plant, and the highest fresh and dry weights of plants were obtained from the treatment of biofertilizer plus a half dose of chemical fertilizer. The lowest fresh and dry weights of plants occurred with the 50% NPK (Mahfouz and Sharaf-Eldin, 2007).

Adathoda vasica plants inoculated with different isolates of *A. chroococcum* revealed significantly increased nitrogen content in shoot compared to the control plants. Similarly, the root nitrogen content was also significantly higher in *A. chroococcum* inoculated plants compared to control plants (Anantha, 2007).

The co-inoculation of mulberry with phosphate solubilizing micro-organisms (*Bacillus megaterium*), nitrogen fixing bacteria (*A. chroococcum*) and arbuscular mycorrhiza (*Glomus fasciculatum*) has influenced its macronutrient uptake through leaf. The data revealed that maximum nitrogen, phosphorus and potassium uptake through leaf has taken place due to co-inoculation treatments as compared to the un inoculated treatments (Baqual and Das, 2006).

This research to study the influence of different biofertilizers either as N-fixing or P dissolving bacteria (PDB) on the soil microbiological properties and the wheat production in new cultivated sandy soil. The traditional organic manuring with farmyard was used as a base treatment, while two bacterial strains were used either

individually or in combination together. The order of strain influences on crop yield and bacterial count arranged as follows mixed treatment with both microorganisms gave the highest response but the lowest effects were recorded in the control. Azotobacters seemed to be specified in enhancing grain production and all growth parameters either individually or combined with phosphate dissolving bacteria **(**Abd El-Gawad & Zeinab, 2006).

The aim of this research was to investigate the effect of inoculation (*A. chroococcum* and *actinomycetes*) and nitrogen mineral fertilizers on the yield of wheat and on the number and activity of certain microorganisms in rhizospheric soil. Depending on the variety and type of treatment, the increase of yield was 8-11% (Mirjana *et al*, 2006).

The effect of biofertilizers (*Azotobacter* and *Azospirillum*) and synthetic fertilizers (urea) were studied separately and in different combinations to establish morphological, biochemical, yield and biomass effects of *Echinochloa frumentacea*. Both bacterial inoculants at all levels and combination of chemical nitrogen show an increase in growth, yield and biochemical components when compared to the control. Biofertilizers with 100% urea treatment produced highest yields compared control. When compared the *Azospirillum* and *Azotobacter* combinations, *Azospirillum* along with 100% urea yielded better results than control **(**Chandrasekar *et al*, 2005).

The present investigation was carried out during two successive winter seasons (2002-2003 & 2003-2004). It studies the effect of bio-fertilizers (*A. chroccocum & phosphorein*) singly or in combination with different rates of N and P chemical fertilizers on growth, yield, sex ratio, seeds (yield & quality) of spinach plants *cv. Dokki*. Seeds inoculation with biofertilizers (*Azotobacter & phosphorein*) enriched the plant rhizosphere with such microorganisms compared with un-inoculated control. Application of phosphorein increased plant fresh yield by 27.2 and 42.3% and 16.3 and 10.4% in seed yield over the control in the first and second seasons, respectively (El-Assiouty & Abo-Sedera, 2005).

The usage of the bacteria containing fertilizers and the wood ash correspond the criteria of environmental friendly nutrient supply. The objective of this study was to evaluate the effects of three bio-fertilizers containing a living and dead algae, N- fixer (*A. chroococcum*) and P–solubilizer (*Bacillus megaterium*) on the growth of plants.

The use of bio-fertilizer resulted in the highest biomass and increased the nutrient uptake by plants. The percentage and the vigour of germination were 10-30% higher than control values (Marianna *et al*, 2005).

Three species of Azotobacter, viz., *A. chroococcum*, *A. vinelandii* and *A. beijerinckii* were isolated, purified and identified. These species exhibit high growth, nitrogen fixation and in vitro production of phytohormone (IAA) at NaCl salinity of 30 g l^{-1}. The azotobacters, which were inoculated with Rhizophora seedlings, increased significantly the average root biomass up to by 98.2%, the root length by 48.45%, the leaf area by 277.86%, the shoot biomass by 29.49% as compared to controls and they also increased the levels of total chlorophylls and carotenoids up to by 151.0% and 158.73%, respectively (Ravikumara *et al,* 2004).

The objective of this greenhouse study was to evaluate the effects of four biofertilizers containing an arbuscular mycorrhizal fungus (*Glomus mosseae* or *Glomus intraradices*) with or without N-fixer (*A. chroococcum*), P solubilizer (*Bacillus megaterium*) and K solubilizer (*Bacillus mucilaginous*) on soil properties and the growth of Zea mays. The use of (*G. mosseae* and three bacterial species) resulted in the highest biomass and seedling height. This greenhouse study also indicated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. Microbial inoculum not only increased the nutritional assimilation of plant (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil (Wua *et al,* 2004).

Seed inoculation of wheat varieties with P solubilizing and phytohormone producing *A. chroococcum* showed better response compared with controls. Mutant strains of *A. chroococcum* showed higher increase in grain (12.6%) and straw (11.4%) yield over control and their survival (12-14%) in the rhizosphere as compared to their parent soil isolate (P4). Mutant strain M37 performed better in all three varieties in terms of increase in grain yield (14.0%) and root biomass (11.4%) over control (Vivek *et al*, 2004).

The effect of inoculation of vermicompost with nitrogen-fixing *A. chroococcum* strains, *Azospirillum lipoferum* and the phosphate solubilizing *Pseudomonas striata* on N and P contents of the vermicompost was assessed. Inoculation of N2 Fixing bacteria into

vermicompost increased contents of N and P. Enriching vermicompost with rock phosphate improved significantly the available P when inoculated with P. striata. During the incubation period, the inoculated bacterial strains proliferated rapidly, fixed N and solubilized added and native phosphate (Vivek and Singh, 2001).

Single or dual inoculation of wheat seedlings (*Triticum aestivum L. cv.* Sakha 69) with *A. chroococcum, Azospirillum brasilense* or *Streptomyces mutabilis* in sterilized soil resulted in significant stimulation of their populations in the rhizosphere, compared with the initial values. Single and dual inoculations stimulated plant growth, significantly increased the concentrations of indole-3-acetic acid (IAA), P, Mg, N and total soluble sugars (TSS) in wheat shoots. Soil content of N increased by single inoculation with Azotobacter and all dual inoculations (El-Shanshoury, 1995).

Nitrogen-fixing bacteria belonging to the genus Azotobacter and *Azospirillum* have been used as nitrogenous fertilizers in some crops, such as tomatoes, potatoes and sugar beets, resulting in a substantial increase in yield after a short period of time. Strains of Azotobacter (*vinelandii* and *chroococcum*) and *Azospirillum brasilense* are extremely efficient as N_2-fixing bacteria. Their use substantially increases the yield in many agricultural products and eliminates the need for nitrogenous fertilizers (Martin *et al*, 1993).

Larger populations of bacteria and actinomycetes were recovered from the rhizospheres of tomato plants inoculated with the mycorrhizal fungus *Glomus fasciculatus* and *Azotobacter chroococcum*, either individually or together, than from those of non-inoculated plants. The dry weights of tomato plants inoculated with both *G. fasciculatus* and *Azotobacter chroococcum* were significantly (62%) greater than non-inoculated plants. These results suggest a synergistic or additive interaction between *Glomus fasciculatus* and *Azotobacter chroococcum (*Bagyaraj & Menge, 1978*).*

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Chemicals

Table 3.1 List of chemicals used in this study.

$FeCl_3 \cdot 6H_2O$	Glucose
Crystal Violet	K_2HPO4
Safranine	KH_2PO4
Acetone-Alcohol	NaCI
Chemical fertilizer	$MgSO_4 . 7H_2O$
Compost	$CaSO_4.2H_2O$
Distilled Water	$NaMoO4.2H_2O$
Agar Agar	$FeSO_4$
	$CaCO_3$

3.1.2 Instruments

Table 3.2 List of equipments used in this study.

Manufacter / country	**Instrument**
Heraeus (Germany)	Incubator
LW. Scientific (USA)	Microscope
Selecta (Spain)	Refrigerator
Selecta (Spain)	Ph Meter
Chromatic (India)	Spectrophotometer
Boxun (China)	Autoclave
ADAM (UK)	Balance
Heraeus (Germany)	Shaker
N-Bioteck (Korea)	Oven
Biomega (USA)	Hotplate

3.1.3 Media

3.1.3.1 Burks Media 1L

Table 3.3 Culture Media (Burks Media)

Manufacture	Quantity/ g	Chemicals
Applichem (Jermany)	10	Glucose
Applichem (Jermany)	0.64	K_2HPO_4
Applichem (Jermany)	0.16	KH_2PO_4
Frutarom (Zionist enemy)	0.2	NaCl
Himedia (India)	0.2	$MgSO_4$. $7H_2O$
Applichem (Jermany)	0.05	$CaSO_4.2H_2O$
Merch (Jermany)	0.01	$NaMoO_4.2H_2O$
Frutarom (Zionist enemy)	0.003	$FeSO_4$

3.1.3.2 Starch Agar Media (Himedia- India)

- Starch
- Animal 5g/L
- Starch Soluble 2g/L
- Meat Extact 3g/L
- Agar 15g/L

3.1.4 Organisms - *Azotobacter chroococcum*

The bacterium used in this experiment was *A. chroococcum*. This microorganism was isolated locally from the roots of *Zea mays* culture in bietlahya.

3.1.5 Cucumber Seeds

The seeds were purchased from the seed market (Royal Sluis –Holland).

3.2 Methods

3.2.1 Isolation and Identification of *Azotobacter chroococcum*

3.2.1.1 Collection of Soil Sample

The soil used in this study was taken at 10 - 15cm depth supplied from 3 random place of maize field, bietlahia. Soil samples contained root of *Zea mays*. Soil samples were air dried to be used for isolation of *Azotobacter chroococcum*.

3.2.1.2 Enrichment of *A. chroococcum*

N-free medium for enrichment of *azotobacter* (Burks media). 2g of soil samples were added to 500-ml Erlenmeyer flasks containing 18 ml of Burk's liquid. (Martinez-Toledo, *et al*, 1985). The samples were incubated for 4-7 days at 27-30°C.

3.2.1.3 Isolation and Subculture of Nitrogen-Fixing Bacteria

An aliquot (0.1 ml) of the bacterial suspension growing out (soil and burks media) was spread on the plates of Burk's medium agar. Plates were incubated at 28°C for 3 days. Bacterial colonies were subcultured onto sterile *Azotobacter* agar plates and the plates were incubated at 28°C for 3 days. Typical bacterial colonies were observed over the streak. Well isolated single colony was picked up and re-streaked to fresh *Azotobacter* agar plate and incubated similarly.

3.2.2 Characterization of the Isolated Strain

After 3 days of incubation, different characteristics of colonies such as shape, size, surface, color, pigmentation were recorded. Morphological characteristics of the colony of each isolate were examined on *Azotobacter* agar plates. Production of diffusible and non-diffusible pigments determined on Burk's solid medium after 5 days of incubation at 30°C.

3.2.2.1 Morphological Test

1- **Colony Shape**

Streak a plate of Burks media agar using isolated colonies from 1-2 old media and incubate at 30°C for 1-5 days and notice the colony shape and color .

2- Gram Staining

A drop of sterile distilled water was placed in the center of glass slide. A lapful of growth from young culture was taken, mixed with water, and placed in the center of slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the lower site of the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently in flow of tap water. Then the slide was flooded with iodine solution, immediately drained off, and flooded again with Lugal iodine solution. After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95% ethanol. After that, it was washed with water within 15 to 30 s and blot dried carefully. The smear was incubated with safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100X power with oil immersion and data were recorded.

3- Motility Test

Bacteria are introduced into a semisoft agar medium by performing a stab with an inoculating needle. After incubating the tube, motility is determined by examining whether or not the bacteria have migrated away from the stab line and throughout the medium.

3.2.2.2 Starch Hydrolysis

Starch agar is used for cultivating microorganisms being tested for starch hydrolysis. Flood the surface of a 48-hour culture on starch agar with Gram Iodine. Iodine solution (Gram's) is an indicator of starch. When iodine comes in contact with a medium containing starch, it turns blue. If starch is hydrolyzed and starch is no longer present, the medium will have a clear zone next to the growth.

3.2.3 Preparation of Bacterial Suspensions for Seeds Inoculation

The bacterial inoculants were prepared where a loopful of the respective *A. chroococcum* isolate was transferred to 2 ml of the burks liquid medium and incubated overnight then transferred into 50 ml burks liquid medium and incubated for 7 days on a rotary shaker. Turbidity, as bacterial growth indicator, of the cultures was adjusted calorimetrically to optical density of 1.6 at wavelength of 420 nm, or the

bacteria was grown on nitrogen-free media and incubated at 28˚C for 5 days until early log phase.

3.2.4 Pot Experiment

The present investigation was carried out during the season of (2009/2010) at greenhouse at Gaza strip. The experiment consisted of seven treatments of chemical, organic and biofertilizers arranged in a complete randomized blocks design with thirty replicates for each treatment and 2 seeds were transplanted in each pot (after germination one of two seeds is disposed), which mean that each treatment had 60 seeds, the treatments as shown below:

A = Control (no inoculation).

B = Biofertilizer only (*A. chroococcum*).

C = Organic only (compost).

D = Chemical fertilizer only.

E = Organic + Biofertilizer (*A. chroococcum*)..

F = Biofertilizer + 20% Chemical fertilizer.

G = Biofertilizer (two doses of *A. chroococcum*).

The total number of seeds were 420 seeds. All seeds were sowing in 210 pots (d = 20cm, h = 30cm), these pots were distributed in completely randomized design. There were five arrows, each one have the 7 treatment (A,B,C,D,E,F,G) distributed randomly, where each treatment have 6 pots in each arrow.

So 240 seeds were inoculated with *A. chroococcum*, 60 seeds as control, 60 seeds with organic, and 60 seeds with chemical fertilizer.

Figure 3.1 Green house arrangement

The soil: The basic properties of the soil used for this pot experiment were as follows: sand = 58.84%, silt = 1.72%, clay = 29.44%, with pH = 7.3, EC = 540 mg/L.

3.2.5 Inoculation of the Seeds

The seeds were inoculated immediately before sowing, 240 of cucumber seeds (biofertilizer, organic + biofertilizer, biofertilizer + 20% chemical fertilizer, biofertilizer (two doses)) were placed in bacterial suspensions for one hour before sowing under sterilized conditions and then transferred to unsterilized soil, where the other 180 seeds (control, compost, chemical) were placed in burks media (without sucrose).

The sowing of seeds were at 17-11-2009 and it continue up to the mid of february of 2010. After the plants were harvested, the following data were recorded at flowering stages and fruiting stage of cucumber plant.

3.2.6 The Growth Parameters

The next parameters, plant height (cm), number of branches, stem wet weight (g), root wet weight (g), stem dry weight (g), root dry weight (g) were measured. Amount of nitrogen (%) of shoot and root, were measured by automated kjeldahl method.

3.2.7 Statistical Analysis

The data were analyzed statistically by SPSS analysis (version 13).

Chapter 4

Results

4.1 Isolation and Identification of *Azotobacter chroococcum*

We succeed to isolate a kind of bacteria that can fix nitrogen by using of N-free medium (Burks media).

4.1.1 Isolation and Subculture of Nitrogen-Fixing Bacteria.

4.1.2 Characterization of the Isolated Strain

The isolated bacteria was characterized by morphological and biochemical tests.

4.1.2.1 Morphological Tests

1- Colony Shape at Burks media.

Colonies are moderately slimy, turning black or black-brown on aging as in Figure 4.1. The pigment produced is water-undiffusible.

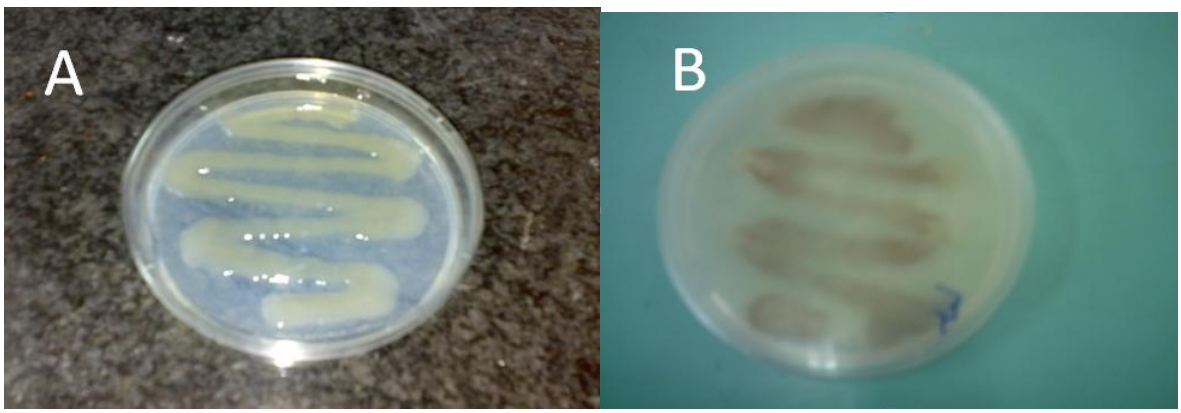

Figure 4.1 Colonies morphoogy at Burks media, A, morphoogy at new culture, B, old culture with black-brown pigments

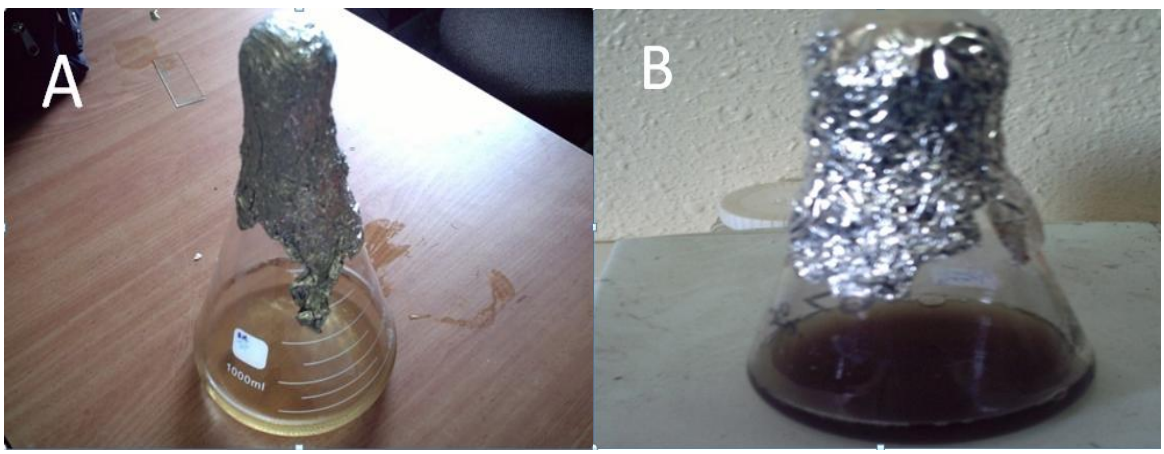

Figure 4.2 The pigments of *A.chroococcum,* A, first days of inoculation, B, after 5 days of inoculation.

2- Gram's Staining

Gram negative, cells of *A. chroococcum* are pleomorphic, bluntly rod, oval, or coccus shaped. The cell shape changes dramatically in time or with changes in growth (medium) conditions. Cells are often in pairs see Figure 4.3.

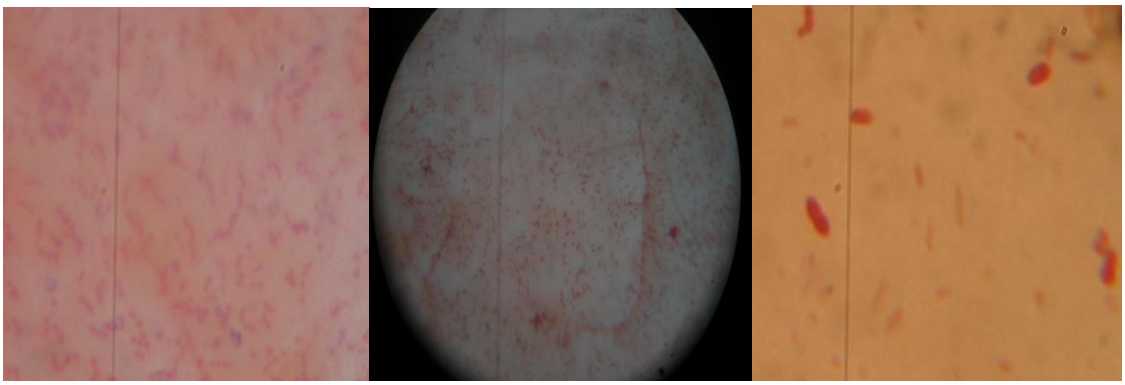

Figure 4.3 Gram negative, cells of *A. chroococcum*, cells are often in pairs.

3- Motility Test

As shown in figure 4.4 the bacteria have migrated away from the stab line and throughout the medium

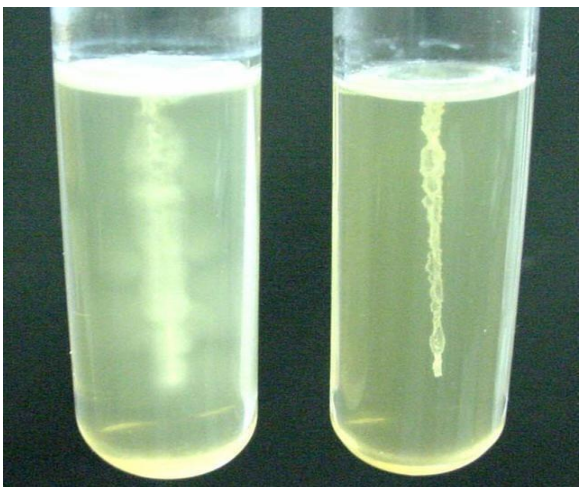

Figure 4.4 The tube at the left show positive motility test

4.1.2.2 Starch Hydrolysis

By pouring Gram's iodine over the growth on the medium, there were a clear zone next to the growth see Figure 4.5.

Figure 4.5 Positive starch hydrolysis

4.2 Bacterial Suspensions for Seeds Inoculation

Figure 4.6 After 5 days of inoculation

4.3 Statistical Analysis

4.3.1 Lengths of Cucumber

Table 4.1 and figure (4.7) show the mean of the final length of shoot. The mean of the final length of shoot of chemically treated plants is higher than that of all other treatments. The mean of B is higher than A, where F is higher than E, C, G and B. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001), compared to control and not significant in all other treatments (table 4.2).

Table (4.1) Mean and standard deviation for the final length of shoot.

Standard deviation	Mean/cm	Number	Treatments
36.06	106.70	30	A - control
27.89	114.13	30	B - Biofertilizer (one dose)
33.33	105.00	30	C - Organic
27.56	135.33	30	D - Chemical
27.17	110.33	30	E - Organic + Biofertilizer
25.52	120.63	30	F - 20% Chemical + Biofertilizer
32.44	104.20	30	G - Biofertilizer (two dose)
31.52	113.76	210	Total

Table (4.2) Comparison of the final length of shoot for different treatments:

P value	Mean difference(I-J)	(J) different variables	(I)
0.342	-7.433	B = biofertilizer only.	A –control
0.828	1.700	C = organic only (compost).	
0.001	-28.633	D = chemical fertilizer only.	
0.642	-3.633	E = organic + biofertilizer.	
0.076	-13.933	F = biofertilizer + 20% chemical.	
0.749	2.500	G = biofertilizer (two doses).	
0.342	7.433	A = control.	B - Biofertilizer
0.243	9.133	C = organic only (compost).	
0.007	-21.200	D = chemical fertilizer only.	
0.627	3.800	E = organic + biofertilizer.	
0.406	-6.500	F = biofertilizer + 20% chemical.	
0.204	9.933	G = biofertilizer"(two doses).	

0.828	-1.700	A = control.	C - Organic
0.243	-9.133	B = biofertilizer only.	
0.001	-30.333	D = chemical fertilizer only.	
0.002	-5.333	E = organic + biofertilizer.	
0.061	-15.633	F = biofertilizer + 20% chemical.	
0.001	0.800	G = biofertilizer"(two doses).	
0.001	28.633	A = control.	D - Chemical
0.007	21.200	B = biofertilizer only.	
0.001	30.333	C = organic only (compost).	
0.002	25.000	E = organic + biofertilizer.	
0.061	14.700	F = biofertilizer + 20% chemical.	
0.001	31.133	G = biofertilizer (two doses).	
0.642	3.633	A = control.	E - Organic + Biofertilizer
0.627	-3.800	B = biofertilizer only.	
0.495	5.333	C = organic only (compost).	
0.002	-25.000	D = chemical fertilizer only.	
0.188	-10.300	F = biofertilizer + 20% chemical.	
0.433	6.133	G = biofertilizer (two doses).	
0.076	13.933	A = control.	F - 20% Chem. + Biofertilizer
0.406	6.500	B = biofertilizer only.	
0.046	15.633	C = organic only (compost).	
0.061	-14.700	D = chemical fertilizer only.	
0.188	10.300	E = organic + biofertilizer.	
0.036	16.433	G = biofertilizer (two doses).	
0.749	-2.500	A = control.	G - Biofertilizer (two dose)
0.204	-9.933	B = biofertilizer only.	
0.918	-0.800	C = organic only (compost).	
0.001	-31.133	D = chemical fertilizer only.	
0.433	-6.133	E = organic + biofertilizer.	
0.036	-16.433	F = biofertilizer + 20% chemical.	

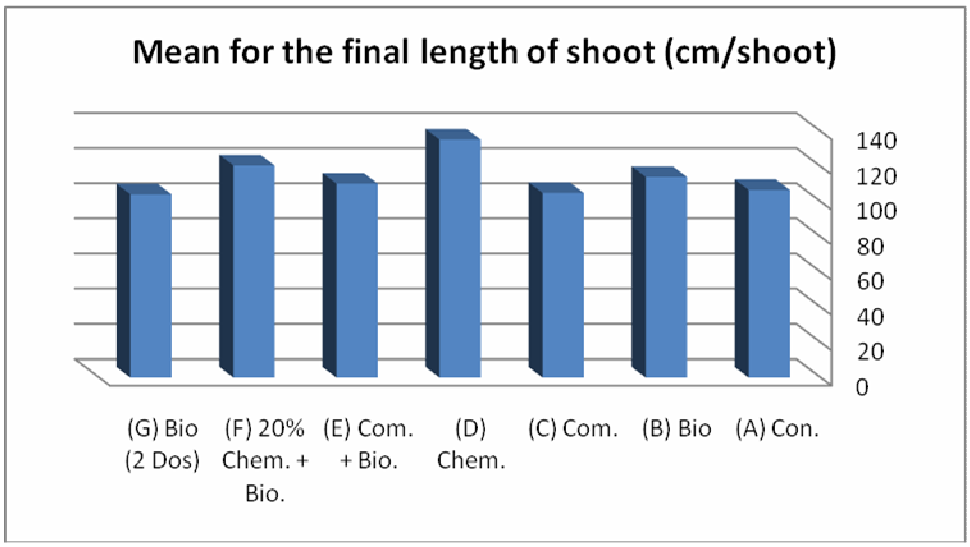

Figure 4.7 Mean for the final length of shoot

Table (4.3) shows the mean of the length of root. The mean of the length of root of biofertilizer treated plants B is higher than that of all other treatments. The mean of B is higher than all treatments. The mean difference is statistically not significant in the case of all treatments.

Table (4.3) Mean and standard deviation for the root length.

Standard deviation	Mean/cm	Number	Treatments
15.48	45.00	30	A - control
11.93	52.23	30	B - Biofertilizer (one dose)
15.44	44.00	30	C - Organic
13.05	43.63	30	D - Chemical
15.47	43.60	30	E - Organic + Biofertilizer
22.41	50.60	30	F - 20% Chemical + Biofertilizer
18.50	51.57	30	G - Biofertilizer (two dose)
16.55	47.23	210	Total

Table (4.4) Comparison of the root length for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.089	-7.233	B = biofertilizer only.	A – control
0.813	1.00	C = organic only (compost).	
0.747	1.36	D = chemical fertilizer only.	
0.741	1.40	E = organic + biofertilizer.	
0.187	-5.60	F = biofertilizer + 20% chemical.	
0.122	-6.56	G = biofertilizer (two doses).	

0.089	7.23	A = control.	B - Biofertilizer
0.053	8.23	C = organic only (compost)	
0.043	8.63	D = chemical fertilizer only.	
0.042	8.63	E = organic + biofertilizer	
0.700	1.63	F = biofertilizer + 20% chemical.	
0.875	0.666	G = biofertilizer (two doses).	
0.813	-1.00	A = control.	C - Organic
0.053	-8.23	B = biofertilizer only.	
0.931	0.366	D = chemical fertilizer only.	
0.925	0.400	E = organic + biofertilizer.	
0.120	-6.96	F = biofertilizer + 20% chemical.	
0.075	-7.56	G = biofertilizer (two doses).	
0747	-1.366	A = control.	D - Chemical
0.043	-8.60	B = biofertilizer only.	
0.931	-.366	C = organic only (compost).	
0.994	0.033	E = organic + biofertilizer	
0.101	-6.96	F = biofertilizer + 20% chemical.	
0.062	-7.93	G = biofertilizer (two doses).	
0.741	-1.400	A = control.	E - Organic + Biofertilizer
0.042	-8.63	B = biofertilizer only.	
0.925	-0.40	C = organic only (compost)	
0.994	-0.03	D = chemical fertilizer only.	
0.099	-7.00	F = biofertilizer + 20% chemical.	
0.061	-7.966	G = biofertilizer (two doses).	
0.187	5.600	A = control.	F - 20% Chem. + Biofertilizer
0.700	-1.63	B = biofertilizer only.	
0.120	6.60	C = organic only (compost).	
0.101	6.96	D = chemical fertilizer only.	
0.099	7.00	E = organic + biofertilizer.	
0.819	-0.96	G = biofertilizer (two doses).	
0.122	6.56	A = control.	G – Biofertilizer (two dose)
0.875	-0.666	B = biofertilizer only.	
0.075	7.56	C = organic only (compost).	
0.062	7.93	D = chemical fertilizer only.	
0.061	7.96	E = organic + biofertilizer.	
0.819	0.966	F = biofertilizer + 20% chemical.	

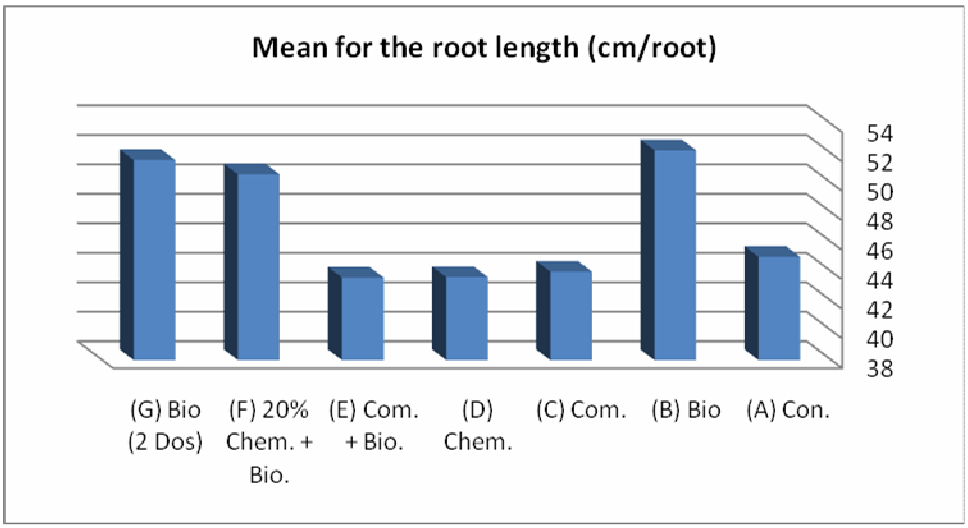

Figure 4.8 Mean for the final length of root

4.3.2 Dry Weights of Cucumber

Table (4.5) and figure (4.9**)** show the means of the weight of dry root. The mean of the dry root weight of chemically treated plants is higher than that of all other treatments . The mean of B is higher than A ,and equal to C, F, G, E. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001) and B, C, F compared to control and not significant in E, G (table 4.6).

Table (4.2) Mean and standard deviation for the weight of dry root.

Standard deviation	Mean/g	Number	Treatments
0.25	0.60	30	A - control
0.40	0.78	30	B - Biofertilizer (one dose)
0.36	0.77	30	C - Organic
0.28	1.08	30	D - Chemical
0.28	0.72	30	E - Organic + Biofertilizer
0.24	0.78	30	F - 20% Chemical + Biofertilizer
0.26	0.78	30	G - Biofertilizer (two dose)
0.33	0.78	210	Total

Table (4.6) Comparison of the weight of dry root for different experiments:

P value	Mean difference (I-J)	(J) different variables	(I)
0.040	-0.180	B = biofertilizer only.	A – control
0.030	-0.170	C = organic only (compost).	
0.001	-0.480	D = chemical fertilizer only.	
0.120	-0.120	E = organic + biofertilizer	
0.020	-0.180	F = biofertilizer + 20% chemical.	
0.110	-0.130	G = biofertilizer (two doses).	
0.040	0.182	A = control	B - Biofertilizer
0.899	0.009	C = organic only (compost).	
0.001	-0.300	D = chemical fertilizer only.	
0.613	0.060	E = organic + biofertilizer.	
0.933	0.003	F = biofertilizer + 20% chemical.	
0.642	0.056	G = biofertilizer (two doses).	
0.029	0.173	A = control	C - Organic
0.899	-0.009	B = biofertilizer only.	
0.001	-0.313	D = chemical fertilizer only.	
0.527	0.055	E = organic + biofertilizer	
0.966	-0.006	F = biofertilizer + 20% chemical.	
0.555	0.046	G = biofertilizer"(two doses).	
0.001	0.486	A = control.	D - Chemical
0.001	0.303	B = biofertilizer only.	
0.001	0.313	C = organic only (compost).	
0.001	0.368	E = organic + biofertilizer.	
0.001	0.307	F = biofertilizer + 20% chemical.	
0.001	0.360	G = biofertilizer (two doses).	
0.119	0.118	A = control.	E – Organic + Biofertilizer
0.613	-0.065	B = biofertilizer only.	
0.527	-0.055	C = organic only (compost).	
0.001	-0.368	D = chemical fertilizer only.	
0.555	-0.061	F = biofertilizer + 20% chemical.	
0.966	-0.008	G = biofertilizer"(two doses).	
0.032	0.179	A = control.	F - 20% Chem. + Biofertilizer
0.933	-0.003	B = biofertilizer only.	
0.966	0.006	C = organic only (compost)	
0.001	-0.307	D = chemical fertilizer only.	

0.555 0.583	0.061 0.052	E = organic + biofertilizer G = biofertilizer (two doses).	
0.110 0.642 0.555 0.001 0.966 0.583	0.126 -0.056 -0.046 -0.360 0.009 -0.052	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. E = organic + biofertilizer. F = biofertilizer + 20% chemical.	G - Biofertilizer (two dose)

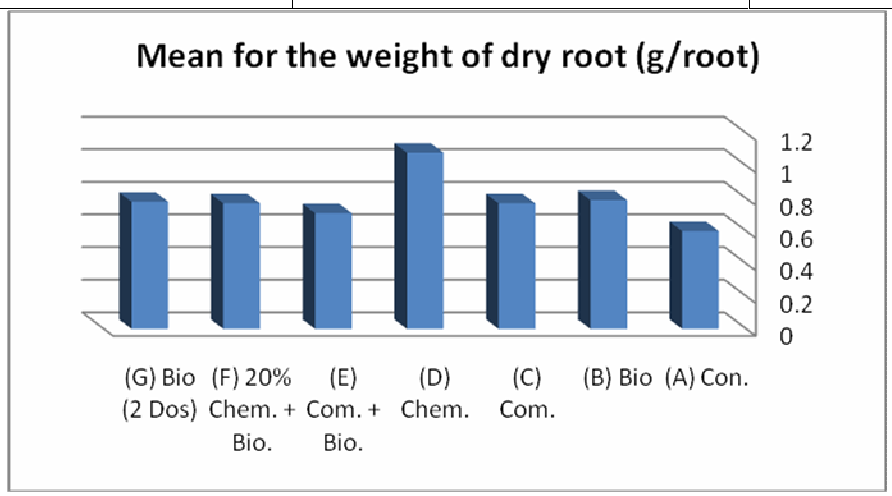

Figure 4.9 Mean for the weight of dry root

Table (4.7) and figure (4.10) show the means of the dry shoot weights. The mean of the dry shoot weight of chemically treated plants is higher than that of all other treatments. The mean of B is higher than A, and lower than C, F, E. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001) compared to control and not significant in all other treatment (table 4.8).

Table 4.7 Mean and standard deviation for the weight of dry shoot

Standard deviation	Mean/g	Number	Treatments
5.00	13.4	30	A - control
6.23	14.68	30	B - Biofertilizer (one dose)
6.23	16.27	30	C - Organic
5.72	24.32	30	D - Chemical
5.72	16.79	30	E - Organic + Biofertilizer
6.55	16.27	30	F - 20% Chemical + Biofertilizer
7.88	14.99	30	G - Biofertilizer (two dose)
6.92	16.74	210	Total

Table 4.8 Comparison of the dry shoot weight for different treatments

P value	Mean difference(I-J)	(J) different variables	(I)
0.899	-0.230	B = biofertilizer only.	A – control
0.163	-2.276	C = organic only (compost).	
0.001	-10.327	D = chemical fertilizer only.	
0.086	-2.800	E = organic + biofertilizer.	
0.072	-2.936	F = biofertilizer + 20% chemical.	
0.547	-0.996	G = biofertilizer (two doses).	
0.899	0.230	A = control	B - Biofertilizer
0.205	-2.040	C = organic only (compost)	
0.001	-10.090	D = chemical fertilizer only.	
0.112	-2.569	E = organic + biofertilizer.	
0.094	-2.706	F = biofertilizer + 20% chemical.	
0.634	-0.766	G = biofertilizer (two doses).	
0.163	2.276	A = control.	C - Organic
0.205	2.046	B = biofertilizer only.	
0.001	-8.050	D = chemical fertilizer only.	
0.745	-0.523	E = organic + biofertilizer.	
0.682	-0.660	F = biofertilizer + 20% chemical.	
0.427	1.280	G = biofertilizer (two doses).	
0.001	10.327	A = control.	D - Chemical
0.001	10.096	B = biofertilizer only.	
0.001	8.050	C = organic only (compost).	
0.001	7.527	E = organic + biofertilizer.	
0.001	7.390	F = biofertilizer + 20% chemical.	
0.001	9.330	G = biofertilizer (two doses).	
0.086	2.800	A = control.	E - Organic + Biofertilizer
0.112	2.569	B = biofertilizer only.	
0.745	0.523	C = organic only (compost).	
0.001	-7.527	D = chemical fertilizer only.	
0.932	-0.136	F = biofertilizer + 20% chemical.	
0.264	1.803	G = biofertilizer (two doses).	
0.072	2.936	A = control.	F -20% Chem. +Biofertilizer
0.094	2.706	B = biofertilizer only.	
0.683	0.660	C = organic only (compost).	
0.001	-7.390	D = chemical fertilizer only.	

0.932 0.229	0.136 1.940	E = organic + biofertilizer. G = biofertilizer (two doses).	
0.547 0.634 0.427 0.001 0.264 0.229	0.996 0.766 -1.280 -9.330 -1.803 -1.940	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. E = organic + biofertilizer. F = biofertilizer + 20% chemical.	G – Biofertilizer (two dose)

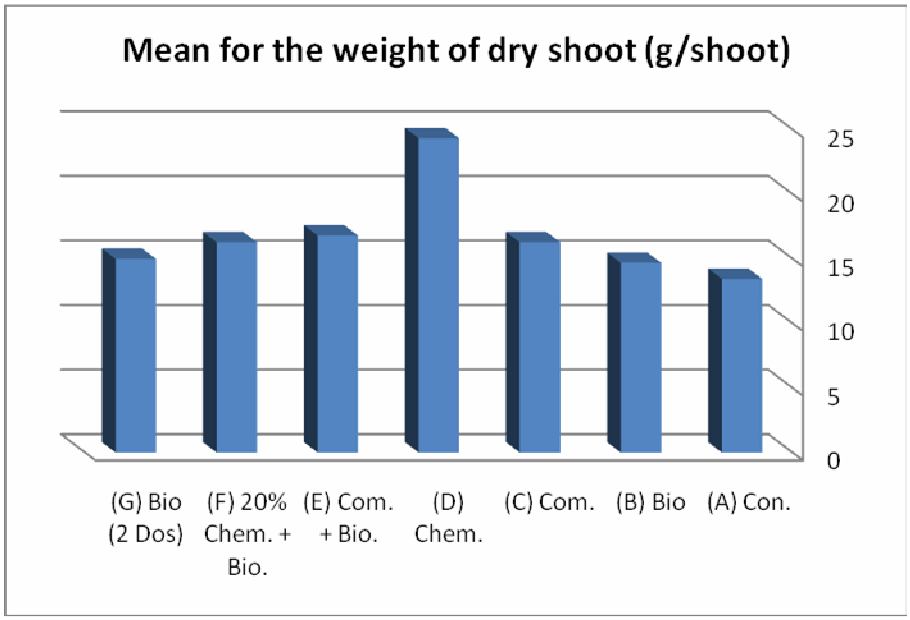

Figure 4.10 Mean for the weight of dry shoot

Table (4.9) and figure (4.11**)** show the means of the dry weights of whole plant. The mean of the dry weight of whole plant of chemically treated plants is higher than that of all other treatments . The mean of B is higher than A and G, and the mean of F is higher than B, C, E, G . The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001) compared to control and not significant in all other treatment (table 4.10).

Table (4.9) Mean and standard deviation for the dry weight of whole plant.

Standard deviation	Mean/g	Number	Treatments
5.29	14.69	30	A - control
6.70	16.33	30	B - Biofertilizer (one dose)
6.36	17.05	30	C - Organic
5.57	25.95	30	D -Chemical
5.70	17.44	30	E - Organic + Biofertilizer
5.93	18.16	30	F - 20% Chemical + Biofertilizer
8.16	15.63	30	G-Biofertilizer (two dose)
7.16	17.64	210	total

Table (4.10) Comparison of the dry weight of whole plant for different experiments:

P value	Mean difference(I-J)	(J) different variables	(I)
0.822	-1.9	B = biofertilizer only.	A – control
0.139	-2.260	C = organic only (compost).	
0.00	-11.160	D = chemical fertilizer only.	
0.077	-2.646	E = organic + biofertilizer.	
0.060	-3.365	F = biofertilizer + 20% chemical.	
0.502	-0.837	G = biofertilizer (two doses).	
0.822	1.9	A = control.	B - Biofertilizer
0.209	-2.317	C = organic only (compost).	
0.001	-11.217	D = chemical fertilizer only.	
0.122	-2.703	E = organic + biofertilizer.	
0.098	-3.422	F = biofertilizer + 20% chemical.	
0.655	-0.895	G = biofertilizer (two doses).	
0.139	2.26	A = control.	C - Organic
0.209	2.317	B = biofertilizer only.	
0.001	-8.90	D = chemical fertilizer only.	
0.772	-.386	E = organic + biofertilizer.	
0.688	-1.105	F = biofertilizer + 20% chemical.	
0.417	1.422	G = biofertilizer (two doses).	
0.001	11.16	A = control.	D - Chemical
0.001	11.22	B = biofertilizer only.	
0.001	8.90	C = organic only (compost).	
0.001	8.51	E = organic + biofertilizer.	

0.001 0.001	7.79 10.32	F = biofertilizer + 20% chemical. G = biofertilizer (two doses).	
0.077 0.122 0.772 0.001 0.911 0.271	2.646 2.703 .386 -8.514 -.719 1.808	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. F = biofertilizer + 20% chemical. G = biofertilizer (two doses).	E - Organic + Biofertilizer
0.060 0.098 0.688 0.001 0.911 0.225	3.365 3.422 1.105 -7.795 .719 2.527	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. E = organic + biofertilizer. G = biofertilizer (two doses).	F - 20% Chem. + Biofertilizer
0.502 0.655 0.417 0.001 0.271 0.225	0.837 0.895 -1.422 -10.322 -1.808 -2.527	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. E = organic + biofertilizer. F = biofertilizer + 20% chemical.	G - Biofertilizer (two dose)

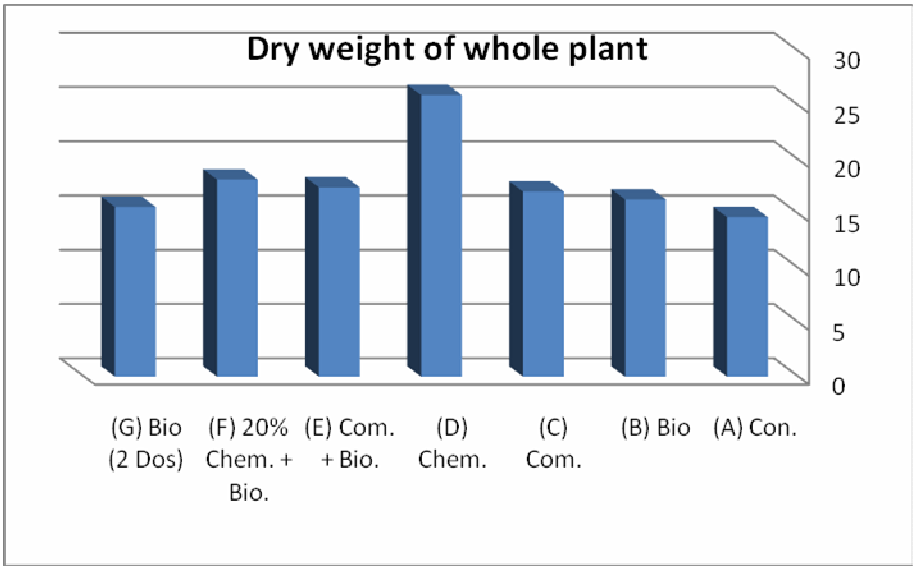

Figure 4.11 Mean for the dry weight of whole plant

4.3.3 Wet Weights of Cucumber

Table 4.11 and figure 4.12 show the means of the wet root weights. The mean of the wet root weight of chemically treated plants is higher than that of all other treatments . The mean of B is higher than A and G, E and equal to C, F. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001) compared to control and not significant in all other treatment (table 4.12).

Table 4.11 Mean and standard deviation for the weight of wet root weights

Standard deviation	Mean/g	Number	Treatments
1.65	5.22	30	A - control
2.55	6.14	30	B - Biofertilizer (one dose)
2.11	6.21	30	C - Organic
1.86	8.68	30	D - Chemical
1.98	5.79	30	E - Organic + Biofertilizer
1.55	6.05	30	F - 20% Chemical + Biofertilizer
2.13	5.14	30	G - Biofertilizer (two dose)
2.26	6.18	210	Total

Table 4.12 Comparison of the weight of wet root for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.077	-0.916	B = biofertilizer only.	A – control
0.056	-0.990	C = organic only (compost).	
0.001	-3.463	D = chemical fertilizer only.	
0.290	-0.573	E = organic + biofertilizer.	
0.110	-0.826	F = biofertilizer + 20% chemical.	
0.882	0.076	G = biofertilizer (two doses).	
0.077	0.916	A = control	B - Biofertilizer
0.887	-0.073	C = organic only (compost).	
0.001	-2.546	D = chemical fertilizer only.	
0.474	0.343	E = organic + biofertilizer.	
0.862	0.090	F = biofertilizer + 20% chemical.	
0.055	0.993	G = biofertilizer (two doses).	

0.056	0.990	A = control	C - Organic
0.887	0.073	B = biofertilizer only.	
0.001	-2.473	D = chemic fertilizer only.	
0.391	0.416	E = organic + biofertilizer	
0.752	0.163	F = biofertilizer + 20% chemical.	
0.040	1.066	G = biofertilizer (two doses).	
0.001	3.463	A = control	D - Chemical
0.001	2.546	B = biofertilizer only.	
0.001	2.473	C = organic only (compost)	
0.001	2.890	E = organic + biofertilizer	
0.001	2.636	F = biofertilizer + 20% chemical.	
0.001	3.540	G = biofertilizer (two doses).	
0.290	0.572	A = control.	E - Organic + Biofertilizer
0.474	-0.343	B = biofertilizer only.	
0.391	-0.416	C = organic only (compost).	
0.001	-2.890	D = chemical fertilizer only.	
0.588	-0.253	F = biofertilizer + 20% chemical.	
0.228	0.649	G = biofertilizer (two doses).	
0.110	0.826	A= control.	F - 20% Chem. + Biofertilizer
0.862	-0.090	B = biofertilizer only.	
0.752	-0.163	C = organic only (compost).	
0.001	-2.636	D = chemical fertilizer only.	
0.588	0.253	E = organic + biofertilizer.	
0.081	0.903	G = biofertilizer (two doses).	
0.882	-0.076	A = control.	G – Biofertilizer (two dose)
0.055	-0.993	B = biofertilizer only.	
0.040	-1.066	C = organic only (compost).	
0.001	-3.540	D = chemical fertilizer only.	
0.228	-0.649	E = organic + biofertilizer.	
0.081	-0.903	F = biofertilizer + 20% chemical.	

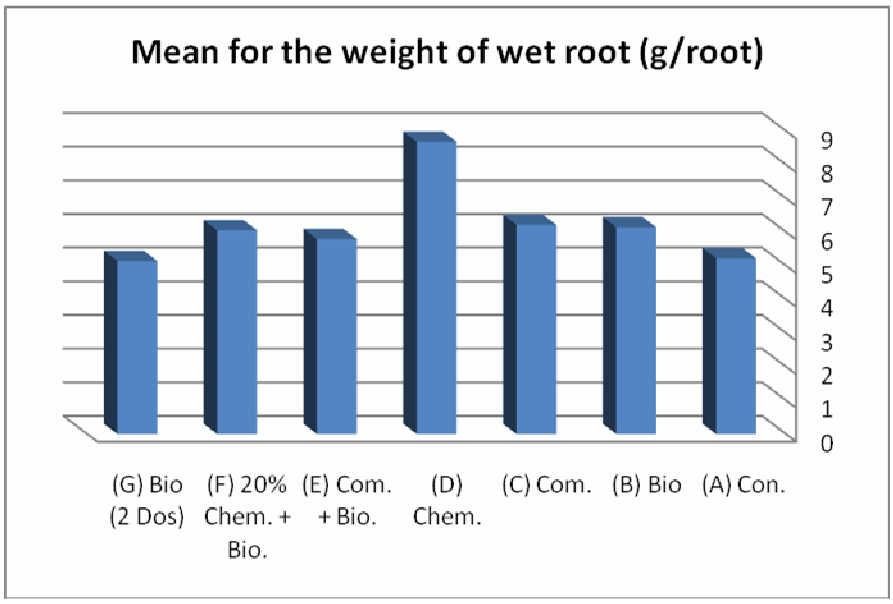

Figure 4.12 Mean for the weight of wet root

Table 4.13 and figure 4.13 show the means of the wet shoot weight. The mean of the wet shoot weight of chemically treated plants is higher than that of all other treatments . The mean of B is higher than A, where F is higher than E, C and B. The mean difference is statistically significant in the case of chemical fertilizer and F treatment (p value = 0.001) compared to control and not significant in all other treatment (table 4.14).

Table 4.13 Mean and standard deviation for the weight of wet shoot.

Standard deviation	Mean/g	Number	Treatments
46.40	111.08	30	A - control
45.71	117.07	30	B - Biofertilizer (one dose)
48.73	121.79	30	C - Organic
46.61	209.15	30	D - Chemical
50.59	128.07	30	E - Organic + Biofertilizer
48.73	144.38	30	F - 20% Chemical + Biofertilizer
55.01	110.59	30	G - Biofertilizer (two dose)
58.06	58.67	210	Total

Table 4.14 Comparison of the wet shoot weight for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.635	-5.996	B = biofertilizer only.	A – control
0.397	-10.713	C = organic only (compost).	
0.001	-98.076	D = chemical fertilizer only.	
0.180	-16.993	E = organic + biofertilizer.	
0.009	-33.303	F = biofertilizer + 20% chemical.	
0.970	0.483	G = biofertilizer (two doses).	
0.635	5.996	A = control.	B - Biofertilizer
0.709	-4.716	C = organic only (compost).	
0.001	-92.080	D = chemical fertilizer only.	
0.385	-10.996	E = organic + biofertilizer.	
0.032	-27.306	F = biofertilizer + 20% chemical.	
0.608	6.480	G = biofertilizer (two doses).	
0.397	10.713	A = control.	C - Organic
0.709	4.716	B = biofertilizer only.	
0.001	-87.363	D = chemical fertilizer only.	
0.385	-6.280	E = organic + biofertilizer.	
0.032	-22.590	F = biofertilizer + 20% chemical.	
0.608	11.196	G = biofertilizer (two doses).	
0.001	98.076	A = control.	D - Chemical
0.001	92.080	B = biofertilizer only.	
0.001	87.363	C = organic only (compost).	
0.001	81.083	E = organic + biofertilizer.	
0.001	64.773	F = biofertilizer + 20% chemical.	
0.001	98.560	G = biofertilizer (two doses).	
0.180	16.993	A = control.	E - Organic + Biofertilizer
0.385	10.996	B = biofertilizer only.	
0.620	6.280	C = organic only (compost).	
0.001	-81.08	D = chemical fertilizer only.	
0.198	-16.31	F = biofertilizer + 20% chemical.	
0.168	17.47	G = biofertilizer (two doses).	
0.009	33.303	A = control.	F - 20% Chem. + Biofertilizer
0.032	27.306	B = biofertilizer only.	
0.75	22.590	C = organic only (compost).	

0.001 0.198 0.008	-64.773 16.310 33.786	D = chemical fertilizer only. E = organic + biofertilizer. G = biofertilizer (two doses).	
0.970 0.608 0.376 0.001 0.168 0.008	-.483 -6.480 -11.196 -98.560 -17.476 -33.786	A = control. B = biofertilizer only. C = organic only (compost) D = chemical fertilizer only. E = organic + biofertilizer. F = biofertilizer + 20% chemical.	G - Biofertilizer (two dose)

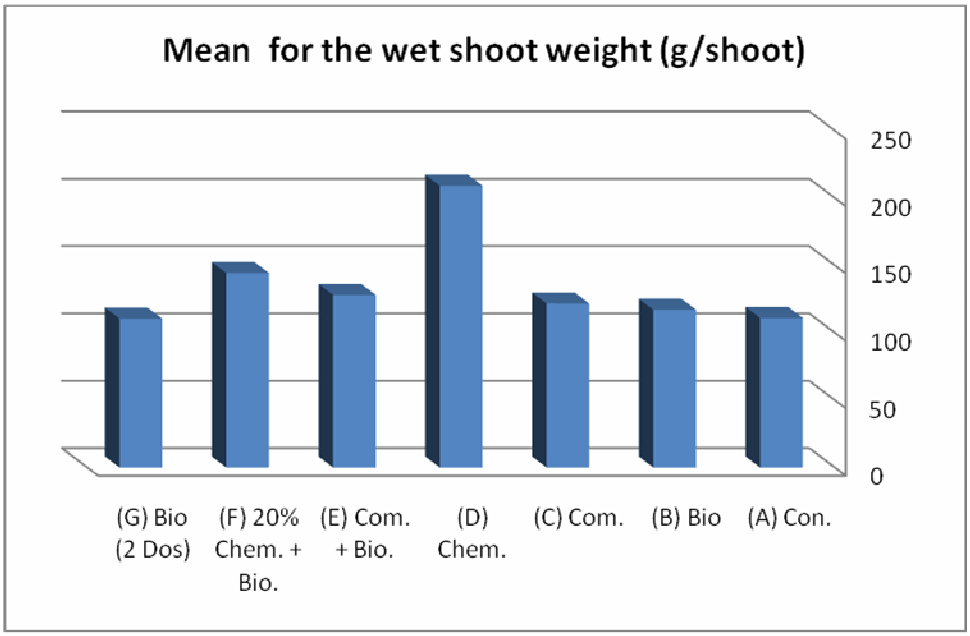

Figure 4.13 Mean for the weight of wet shoot

4.3.4 Different Parameters of Growth of Cucumber.

Throw the 2 month of culture, at the first two week the branches are equal in all treatment, then branches were increased at B, F, E, C, than A, at the end of two month the higher measurement of branches were at B and D (48 and 46 branches respectively).

Table (4.15) shows the mean and the standard deviation of number of branches .The mean of number of branches of chemically treated plants is higher than that of all other treatments. The mean of B is higher than A (which is the least one), C, F, G and equal to E. The mean difference is statistically not significant in the case of all treatments.

Table (4.15) Mean and standard deviation for the number of branches

Standard deviation	Mean	Number	Treatments
6.95	18.15	39	A - control
10.11	20.08	39	B - Biofertilizer (one dose)
10.91	19.67	39	C - Organic
10.74	24.26	39	D - Chemical
8.50	20.00	39	E - Organic + Biofertilizer
8.19	18.33	39	F - 20% Chemical + Biofertilizer
9.29	19.38	39	G - Biofertilizer (two dose)

Table 4.16 Comparison of the number of branches for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.349	-1.940	B = biofertilizer only.	A – control
0.474	-1.480	C = organic only (compost).	
0.004	-6.102	D = chemical fertilizer only.	
0.368	-1.870	E = organic + biofertilizer.	
0.921	-0.205	F = biofertilizer + 20% chemical.	
0.546	-1.250	G = biofertilizer (two doses).	
0.349	1.940	A = control.	B - Biofertilizer
0.824	0.460	C = organic only (compost).	
0.046	-4.150	D = chemical fertilizer only.	
0.970	0.070	E = organic + biofertilizer	
0.402	1.740	F = biofertilizer + 20% chemical.	
0.739	0.690	G = biofertilizer (two doses).	
0.474	1.480	A = control.	C - Organic
0.824	-0.460	B = biofertilizer only.	
0.027	-4.610	D = chemical fertilizer only.	
0.853	-0.380	E = organic + biofertilizer.	
0.537	1.280	F = biofertilizer + 20% chemical.	
0.913	0.230	G = biofertilizer (two doses).	
0.004	6.102	A = control.	D - Chemical
0.046	4.150	B = biofertilizer only.	
0.027	4.610	C = organic only (compost).	
0.043	4.230	E = organic + biofertilizer.	
0.005	5.890	F = biofertilizer + 20% chemical.	
0.020	4.840	G = biofertilizer (two doses).	

0.368	1.870	A = control.	
0.970	-0.070	B = biofertilizer only.	
0.853	0.384	C = organic only (compost).	E - Organic
0.043	-4.230	D = chemical fertilizer only.	+ Biofertilizer
0.423	1.660	F = biofertilizer + 20% chemical.	
0.767	0.615	G = biofertilizer (two doses).	
0.912	0.921	A = control.	
0.402	0.402	B = biofertilizer only.	
0.537	0.537	C = organic only (compost).	F - 20% Chem.
0.005	0.005	D = chemical fertilizer only.	+ Biofertilizer
0.423	0.423	E = organic + biofertilizer.	
0.613	0.613	G = biofertilizer (two doses).	
0.546	1.250	A = control.	
0.739	-0.690	B = biofertilizer only.	
0.912	-0.230	C = organic only (compost).	G - Biofertilizer
0.020	-4.840	D = chemical fertilizer only.	(two dose)
0.767	-0.610	E = organic + biofertilizer.	
0.613	1.050	F = biofertilizer + 20% chemical.	

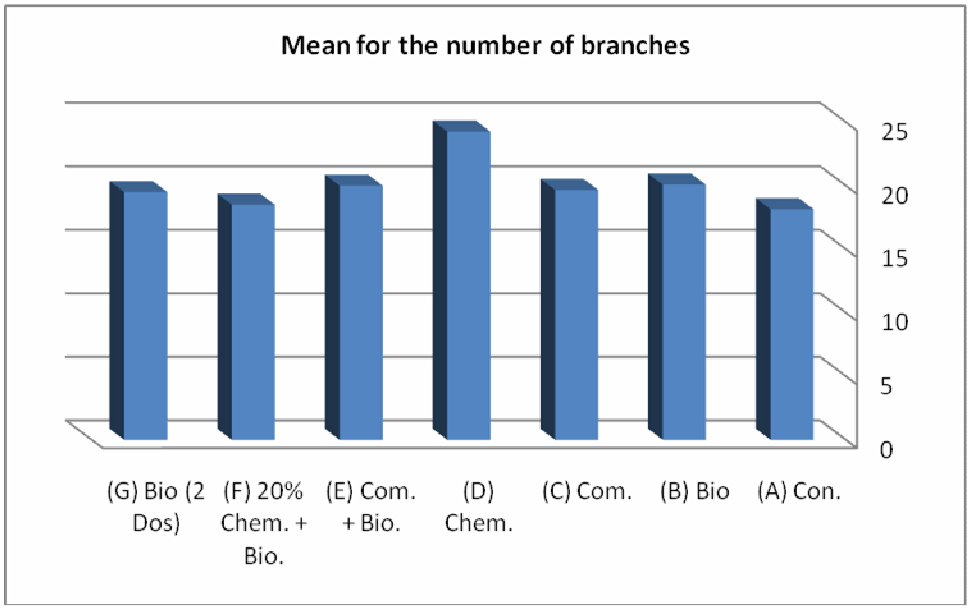

Figure 4.14 Mean for the number of branches

Table (4.17) shows the mean of the length of leave. The mean of the length of leave of chemically treated plants is higher than that of all other treatments. The mean of B, C, E, F and is higher than A. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001) compared to control and not significant in all other treatments (table 4.18).

Table (4.17) Mean and standard deviation for the length of leave

Standard deviation	Mean/g	number	Treatments
1.56	13.62	45	A - control
1.61	14.14	45	B - Biofertilizer (one dose)
1.51	14.38	45	C - Organic
3.29	18.27	45	D - Chemical
2.19	13.66	45	E - Organic + Biofertilizer
2.02	13.83	45	F - 20% Chemical + Biofertilizer
2.23	14.40	45	G - Biofertilizer (two dose)
2.63	14.66	315	Total

Table (4.18) Comparison of the length of leave for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.297	-0.512	B = biofertilizer only.	A – control
0.518	-0.241	C = organic only (compost).	
0.001	-4.130	D = chemical fertilizer only.	
0.124	0.478	E = organic + biofertilizer.	
0.165	0.303	F = biofertilizer + 20% chemical.	
0.728	-0.268	G = biofertilizer"(two doses).	
0.297	0.514	A = control.	B - Biofertilizer
0.092	-0.756	C = organic only (compost).	
0.001	-0.644	D = chemical fertilizer only.	
0.619	-0.036	E = organic + biofertilizer	
0.728	-0.211	F = biofertilizer + 20% chemical.	
0.165	-0.783	G = biofertilizer (two doses).	
0.518	0.241	A = control.	C - Organic
0.092	0.756	B = biofertilizer only.	
0.001	-3.889	D = chemical fertilizer only.	
0.029	0.720	E = organic + biofertilizer	
0.042	0.544	F = biofertilizer + 20% chemical.	
0.766	-0.027	G = biofertilizer (two doses).	

0.001	4.130	A = control.	D - Chemical
0.001	4.644	B = biofertilizer only.	
0.001	3.889	C = organic only (compost).	
0.001	4.609	E = organic + biofertilizer.	
0.001	4.433	F = biofertilizer + 20% chemical.	
0.001	3.862	G = biofertilizer (two doses).	
0.124	-.478	A = control.	E - Organic + Biofertilizer
0.619	0.036	B = biofertilizer only.	
0.029	-0.720	C = organic only (compost).	
0.001	-4.609	D = chemical fertilizer only.	
0.882	-0.175	F = biofertilizer + 20% chemical.	
0.060	-0.747	G = biofertilizer (two doses).	
0.165	-0.303	A = control.	F - 20% Chem. + Biofertilizer
0.728	0.211	B = biofertilizer only.	
0.042	-0.544	C = organic only (compost).	
0.001	-4.433	D = chemical fertilizer only.	
0.882	0.175	E = organic + biofertilizer.	
0.083	-0.571	G = biofertilizer (two doses).	
0.728	0.268	A = control.	G - Biofertilizer (two dose)
0.165	0.783	B = biofertilizer only.	
0.766	0.027	C = organic only (compost).	
0.001	-3.862	D = chemical fertilizer only.	
0.060	0.747	E = organic + biofertilizer.	
0.083	0.571	F = biofertilizer + 20% chemical.	

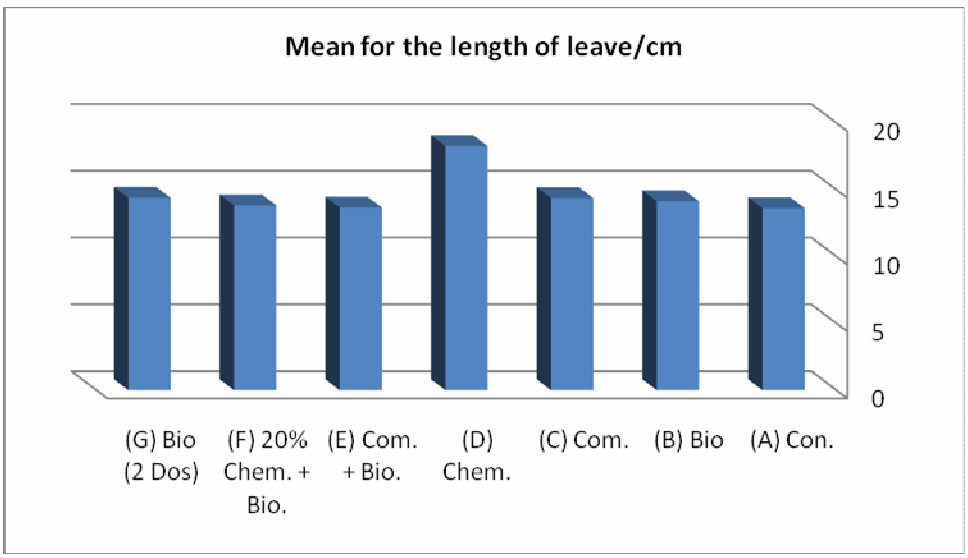

Figure 4.15 Mean for the length of leave.

Table (4.19) shows the mean of the number of leaves. The mean of the number of leaves of chemically treated plants is higher than that of all other treatments. The mean of B is higher than A, F and G, where equal to C. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001), compared to control and not significant in all other treatments (table 4.20).

Table (4.19) Mean and standard deviation for the number of leaves

Standard deviation	Mean	Number	Treatments
4.65	12.39	44	A - control
6.48	15.83	44	B - Biofertilizer (one dose)
6.40	15.07	44	C - Organic
6.35	18.93	44	D - Chemical
6.99	14.77	44	E - Organic + Biofertilizer
6.05	13.75	44	F - 20% Chemical + Biofertilizer
6.61	13.63	44	G - Biofertilizer (two dose)
6.82	14.80	308	Total

Table (4.20) Comparison of the number of leaves for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.054	-3.439	B = biofertilizer only.	A - control
0.058	-2.687	C = organic only (compost).	
0.001	-7.798	D = chemical fertilizer only.	
0.092	-3.479	E = organic + biofertilizer.	
0.334	-2.467	F = biofertilizer + 20% chemical.	
0.376	-2.280	G = biofertilizer (two doses).	
0.054	3.439	A = control.	B - Biofertilizer
0.974	0.752	C = organic only (compost).	
0.007	-4.359	D = chemical fertilizer only.	
0.809	-0.040	E = organic + biofertilizer.	
0.334	0.972	F = biofertilizer + 20% chemical.	
0.296	1.158	G = biofertilizer (two doses).	
0.058	2.687	A = control.	C - Organic
0.974	-0.752	B = biofertilizer only.	
0.007	-5.111	D = chemical fertilizer only.	
0.834	-0.792	E = organic + biofertilizer.	
0.351	0.220	F = biofertilizer + 20% chemical.	
0.311	0.407	G = biofertilizer (two doses).	

0.001	6.54	A = control.	D - Chemical
0.007	3.81	B = biofertilizer only.	
0.007	3.86	C = organic only (compost).	
0.003	4.319	E = organic + biofertilizer.	
0.001	5.331	F = biofertilizer + 20% chemical.	
0.001	5.518	G = biofertilizer (two doses).	
0.092	3.479	A = control.	E - Organic + Biofertilizer
0.809	0.040	B = biofertilizer only.	
0.834	0.792	C = organic only (compost).	
0.003	-4.319	D = chemical fertilizer only.	
0.469	1.012	F = biofertilizer + 20% chemical.	
0.421	1.198	G = biofertilizer (two doses).	
0.334	2.467	A= control.	F - 20% Chem. + Biofertilizer
0.334	-0.972	B = biofertilizer only.	
0.351	-0.220	C = organic only (compost).	
0.001	-5.331	D = chemical fertilizer only.	
0.469	-1.012	E = organic + biofertilizer.	
0.936	0.186	G = biofertilizer (two doses).	
0.376	2.280	A = control.	G - Biofertilizer (two dose)
0.296	-1.158	B = biofertilizer only.	
0.311	-0.407	C = organic only (compost).	
0.001	-5.518	D = chemical fertilizer only.	
0.421	-1.198	E = organic + biofertilizer.	
0.936	-0.186	F = biofertilizer + 20% chemical.	

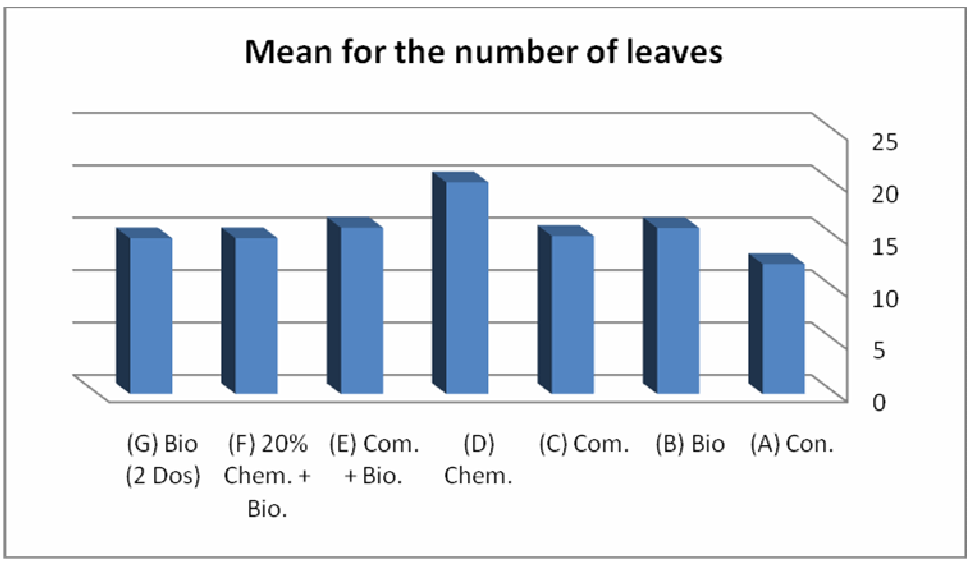

Figure 4.16 Mean for the number of leaves.

4.3.5 Nitrogen Percentage

Table (4.21) and figure (4.17) show means and standard deviations for the shoot nitrogen percentage. The mean of the shoot nitrogen percentage of 20% chemical and biofertilizer treated plants is higher than that of all other treatments. The mean of B is higher than A, C, E, G, where D is higher than B and lower than F. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.002), and in the case of F (p value = 0.001) compared to control and not significant in all other treatments (table 4.22).

Table (4.21) Mean and standard deviation for the shoot nitrogen percentage

Standard deviation	Mean	Number	Treatments
0.20	2.00	3	A - control
0.36	2.30	3	B - Biofertilizer (one dose)
0.20	2.20	3	C - Organic
0.21	2.63	3	D - Chemical
0.00	2.00	3	E - Organic + Biofertilizer
0.10	2.80	3	F - 20% Chemical + Biofertilizer
0.11	2.06	3	G - Biofertilizer (two dose)
0.34	2.28	21	Total

Table (4.22) Comparison of the shoot nitrogen percentage for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.086	-0.30	B = biofertilizer only.	A - control
0.238	-0.20	C = organic only (compost).	
0.002	-0.63	D = chemical fertilizer only.	
1.00	0.00	E = organic + biofertilizer.	
0.001	-0.80	F = biofertilizer + 20% chemical.	
0.688	-0.066	G = biofertilizer (two doses).	
0.086	0.300	A = control.	B - Biofertilizer
0.548	0.100	C = organic only (compost).	
0.059	-0.333	D = chemical fertilizer only.	
0.086	0.300	E = organic + biofertilizer.	
0.008	-0.500	F = biofertilizer + 20% chemical.	
0.173	0.233	G = biofertilizer (two doses).	

0.238 0.548 0.018 0.238 0.002 0.425	0.200 -0.100 -0.433 0.200 -0.600 0.133	A = control. B = biofertilizer only. D = chemical fertilizer only. E = organic + biofertilizer. F = biofertilizer + 20% chemical. G = biofertilizer (two doses).	C - Organic
0.002 0.059 0.018 0.002 0.322 0.004	0.633 0.333 0.433 0.633 -0.166 0.566	A = control. B = biofertilizer only. C = organic only (compost). E = organic + biofertilizer. F = biofertilizer + 20% chemical. G = biofertilizer"(two doses).	D - Chemical
1.000 0.086 0.238 0.002 0.001 0.688	0.000 -0.300 -0.200 -0.633 -0.800 -0.066	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. F = biofertilizer + 20% chemical. G = biofertilizer (two doses).	E - Organic + Biofertilizer
0.001 0.008 0.002 0.322 0.001 0.001	0.800 0.500 0.600 0.166 0.800 0.733	A= control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. E = organic + biofertilizer. G = biofertilizer (two doses).	F - 20% Chem. + Biofertilizer
0.688 0.173 0.425 0.004 0.688 0.001	0.066 -0.233 -0.133 -0.566 0.066 -0.733	A = control. B = biofertilizer only. C = organic only (compost). D = chemical fertilizer only. E = organic + biofertilizer. F = biofertilizer + 20% chemical.	G – Biofertilizer (two dose)

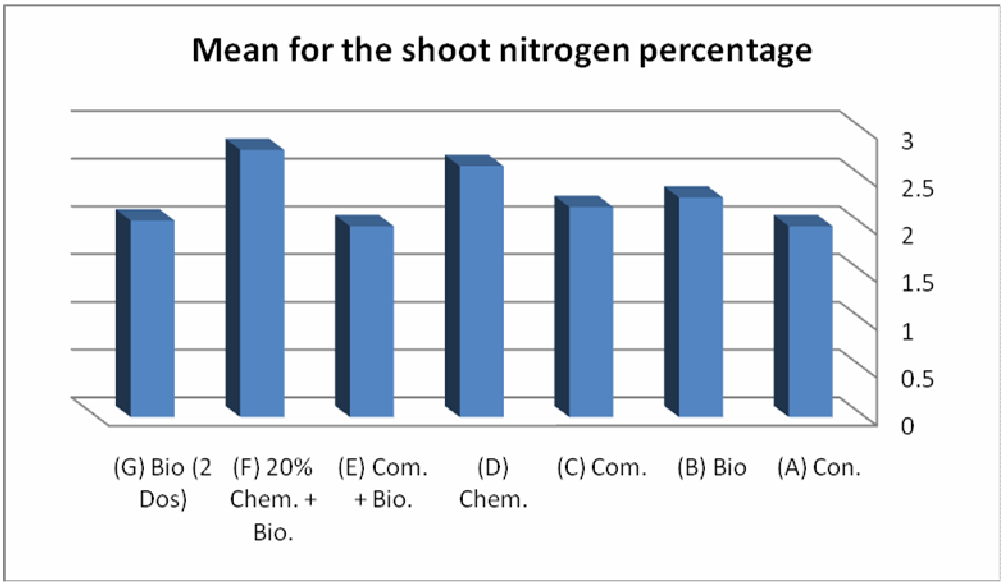

Figure 4.17 Mean for the shoot nitrogen percentage

Table (4.23) shows mean and standard deviation for the root nitrogen percentage. The mean of the number of leaves of chemically treated plants is higher than that of all other treatments. The mean of B is higher than A, C, and equal to E and G, where F is higher than B. The mean difference is statistically significant in the case of chemical fertilizer treatment (p value = 0.001) compared to control and not significant in all other treatments (table 4.24).

Table (4.23) Mean and standard deviation for the root nitrogen percentage

Standard deviation	Mean	number	Treatments
0.05	1.2	3	A - control
0.11	1.5	3	B - Biofertilizer (one dose)
0.20	1.2	3	C - Organic
0.26	2.0	3	D - Chemical
0.10	1.4	3	E - Organic + Biofertilizer
0.17	1.5	3	F - 20% Chemical + Biofertilizer
0.10	1.4	3	G - Biofertilizer (two dose)
0.27	1.5	21	Total

Table (4.24) Comparison of the root nitrogen percentage for different experiments

P value	Mean difference(I-J)	(J) different variables	(I)
0.097	-0.233	B = biofertilizer only.	A - control
0.803	-0.033	C = organic only (compost).	
0.001	-0.766	D = chemical fertilizer only.	
0.184	-0.183	E = organic + biofertilizer.	
0.062	-0.266	F = biofertilizer + 20% chemical.	
0.225	-0.166	G = biofertilizer (two doses).	
0.097	0.233	A = control.	B - Biofertilizer
0.150	0.200	C = organic only (compost).	
0.001	-0.533	D = chemical fertilizer only.	
0.709	0.050	E = organic + biofertilizer.	
0.803	-0.033	F = biofertilizer + 20% chemical.	
0.619	0.066	G = biofertilizer (two doses).	
0.803	0.033	A = control.	C - Organic
0.150	-0.200	B = biofertilizer only.	
0.001	-0.733	D = chemical fertilizer only.	
0.272	-0.150	E = organic + biofertilizer.	
0.097	-0.233	F = biofertilizer + 20% chemical.	
0.327	-0.133	G = biofertilizer (two doses).	
0.001	0.766	A = control.	D - Chemical
0.001	0.533	B = biofertilizer only.	
0.001	0.733	C = organic only (compost).	
0.001	0.583	E = organic + biofertilizer.	
0.002	0.500	F = biofertilizer + 20% chemical.	
0.001	0.600	G = biofertilizer (two doses).	
0.184	0.183	A = control.	E - Organic + Biofertilizer
0.709	-0.050	B = biofertilizer only.	
0.272	0.150	C = organic only (compost).	
0.001	-0.583	D = chemical fertilizer only.	
0.536	-0.083	F = biofertilizer + 20% chemical.	
0.901	0.016	G = biofertilizer (two doses).	

0.062	0.266	A = control.	
0.803	0.033	B = biofertilizer only.	
0.097	0.233	C = organic only (compost).	F - 20% Chem.
0.002	-0.500	D = chemical fertilizer only.	+ Biofertilizer
0.536	0.083	E = organic + biofertilizer.	
0.459	0.100	G = biofertilizer (two doses).	
0.225	0.166	A = control.	
0.619	-0.066	B = biofertilizer only.	
0.327	0.133	C = organic only (compost).	G - Biofertilizer
0.001	-0.600	D = chemical fertilizer only.	(two dose)
0.901	-0.016	E = organic + biofertilizer.	
0.459	0.100	F = biofertilizer + 20% chemical.	

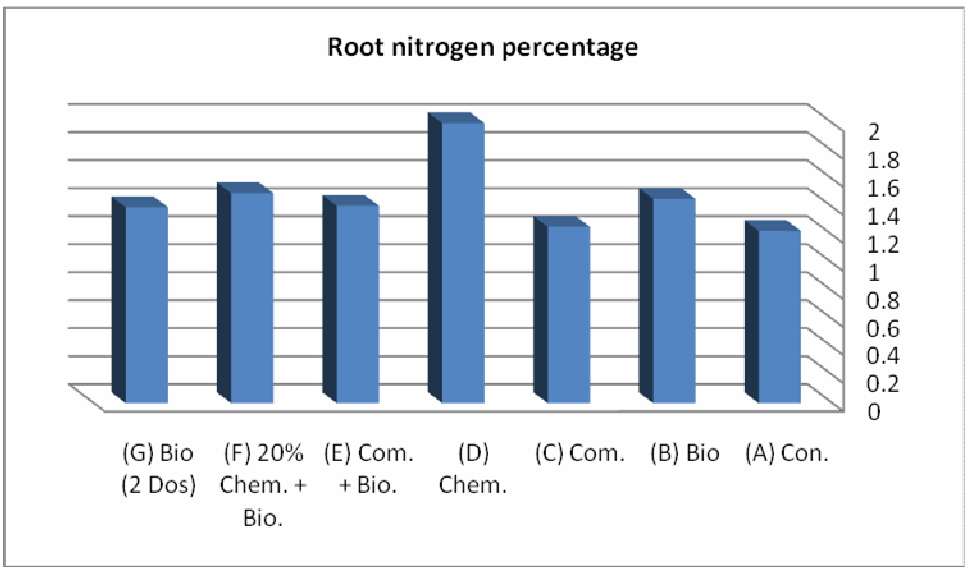

Figure 4.18 Mean for the root nitrogen percentage

4.4 Growth of Cucumber

4.4.1 The Number and Weight of the Last three Collections

As shown in (4.25), control is the least number and weight, then G which were lower than the other treatments, where B is higher than A and G, nearly equal E, F, and lower than C, D, where D is the highest.

Table (4.25) The number and weight of the last three collections of cucumber

Mean	weight of cuccumber	Number of cuccumber	Treatment
55.55	5000g	90	A (control)
58.43	6545g	112	B (biofertilizer)
62.17	7150g	115	C (compost)
64.2	10400g	162	D (chemical)
58.42	7245g	124	E (compost + biofertilizer)
58.72	7164g	122	F (20% chemical + biofertilizer)
55.1	5834g	106	G (biofertilizer two dose)

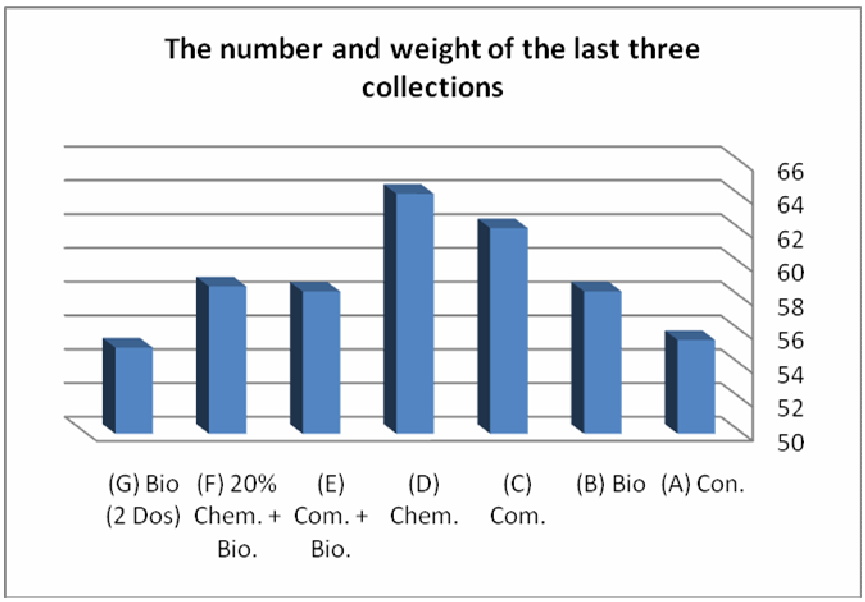

Figure 4.19 The number and weight of the last three collections

4.4.2 Comparison of the Different Parameters

The next table 4.26 show the different between the means of control and the means of biofertilizer for different parameters: as showed all means of biofertilizer, 20% chemical + biofertilizer mean and compost + biofertilizer mean are higher than the means of control which show the activity of *Azotobacter chroococcum* as biofertilizer.

As shown the nitrogen percentage at shoot is the highest at F (20% chem. + bio) where nitrogen percentage at root at B,F, and E is higher than A. It's clear that the treatments B, E, F, in most measurements are nearly equal.

Table (4.26) Comparison of the different parameters means for different experiments

(G)	(F)	(E)	(D)	(C)	(B)	(A)	
0.78	0.77	0.71	1.08	0.77	0.79	0.60	**Dry root weight**
14.99	16.27	16.79	24.32	16.27	14.68	13.4	**Dry shoot weight**
5.14	6.05	5.79	8.68	6.213	6.140	5.22	**Wet root weight**
110.5	144.3	128.0	209.1	121.7	117.0	111.0	**Wet shoot weight**
104.2	120.63	110.3	135.3	105.0	114.1	106.7	**Shoot length**
51.57	50.60	43.60	43.63	44.00	52.23	45.00	**Root length**
156.5	155.1	167.3	178.9	147.9	168.1	147.5	**Length of whole plant**
19.50	18.50	20.00	24.26	19.67	20.08	18.15	**Number of branches**
14.85	14.86	15.86	20.18	15.07	15.83	12.39	**Number of leaves**
1.40	1.5	1.41	2.00	1.26	1.46	1.23	**Root N percentage**
2.06	2.8	2.00	2.63	2.20	2.30	2.00	**shoot N percentage**

Table (4.27) Comparison of the different parameters percentage for different experiments

(G)	(E)	(F)	(D)	(C)	(B)	
30%	16%	28%	80%	28%	31%	**Dry root weight**
7%	20%	14%	70%	14%	9%	**Dry shoot weight**
0.0%	11%	15%	66%	19%	18%	**Wet root weight**
0.0%	15%	44%	88%	9%	5%	**Wet shoot weight**
0.0%	3%	13%	27%	0.0%	7%	**Shoot length**
14%	0.0%	12%	0.0%	0.0%	15%	**Root length**
6%	5%	13%	21%	0.2%	14%	**Length of whole plant**
7%	10%	2%	33%	8%	10%	**Number of branches**
20%	27%	19%	62%	21%	27%	**Number of leaves**
14%	15%	22%	62%	2%	18%	**Root N percentage**
3%	0.0%	40%	31%	10%	15%	**shoot N percentage**

Chapter 5
Discussion

The problem of chemical fertilizers is a global problem, and researchers are working all over the world to find a solution to this problem as it is in the last century, when the chemical fertilizers were first introduced into the agriculture field, most of the problems faced by farmers to increase yield of their plantation have been solved. However, chemical fertilizers slowly started to show their side effect on human and environment (Bin Zakaria, 2009).

The increased use of fertilizers and chemicals have a negative impact on soil quality over time, leading to the accumulation of certain compounds and salts in the soil or transfer such chemicals and salts into the groundwater, which increases the salinity. Gaza Strip is an agricultural land, has a high population density with a small space, and lack of farm land. Farmers use chemical fertilizers in agriculture which caused negative impact on some plants and the environment contributed to the deterioration of biodiversity. In addition, because of fluctuation of rainfall in our country, the effects of chemical fertilizer may be negative in oftentimes, lack of rainfall caused chemicals to accumulate in the soil, lead to low productivity because of the high salinity of the soil due to add fertilizer, where high rainfall caused the descent of chemicals into the groundwater. So due to the fluctuation and irregular rainsfall, the use of fertilizers have many risks.

It should be noted that chemical fertilizers are sometimes difficult to obtain due to the siege as they are costly and have side effects and multiple damages. Moreover the price of chemical fertilizer is expensive and some time not available for farmers (Al-Khiat, 2006). Partial or total replacement of chemical fertilizers will be useful in Gaza Strip to overcome the harmful effects of chemical fertilizers and to maintain soil fertility and groundwater.

Biofertilizers will be the best solution to replace chemical fertilizers. Biofertilizers are the carrier-based preparations containing mainly effective strains of microorganisms in sufficient number, which are useful for nitrogen fixation. Amongst the nutrients, nitrogen is the only nutrient, which play major role in synthesis of chlorophyll, amino

acids and protein building blocks (Mahato *et al*, 2009). Biofertilizers have several advantages over chemical fertilizers, they are non pollutant, in-expensive, utilize renewable resources. In addition to their ability of using free available solar energy, atmospheric nitrogen and water. Beside supplying N_2 to crops, they also supply other nutrients such as vitamins and growth substances (Contra costa, 2003). Amongst biofertilizers, *Azotobacter* strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots. They have been also reported to improve fertility condition of the soil (Mahato *et al*, 2009). Nitrogen-fixer microorganisms such as *A. chroococcum* can supply nitrogen by fixing the nitrogen from atmosphere and convert it into ammonium ion for plants' uptake.

The cucumber, important and desirable to the palestinian consumer, the option is available throughout the year due to cultivation in greenhouses, where the growing season needs to be warm and relatively short. Nitrogen is considered as one of major nutrients required by the plants for growth, development and yield.

The specific objectives of this study was the use of *A. chroococcum* which isolated locally from the soil as a biofertilizer. This study show the role of N-fixing *A. chroococcum* in encouraging plant growth, where using *A. chroococcum* as a biofertilizer stimulates the growth of cucumber, where the use of biofertilizer gave the second best results after chemical fertilizer, and even better than compost, 20% chemical + biofertilizer, and compost + biofertilizer, explains that the bacteria was more effective in nitrogen fixation and supply plant with nitrogen.

There was an excellent growth in plants that were inoculated by bacteria but it's important to indicate that these plants get only the nitrogen while did not get the other nutrients such as potassium and phosphorus, although that growth was clear and in most cases better than the other treatments except plants that took chemical fertilizer where these plants got all the nutrients needed for proper growth.

This indicate that inoculation of *A. chroococcum* had beneficiary response on growth of cucumber.

5.1 Isolation and Identification of *Azotobacter chroococcum*

Azotobacter chroococcum is the most common type of *Azotobacter* presence in the cornfields, and most survival, so used the soil of the cornfields with the use of

appropriate N-free medium, burks medium (which used specifically for *A. chroococcum*), gave us the wanted *A. chroococcum.* Among the other species of *Azotobacter* only *A. chroococcum*, colonies are moderately slimy, semi transparent at first, turning black or black-brown on aging. The pigment produced is not water-diffusible, where the other species of Azotobacter produce water-diffusible pigments, (Figure 4.1).

In our result, the shape, morphology, and the pigments of colony which gave brown color then black-brown on aging, show that the bacteria is *A. chroococcum.* Isolated bacteria were gram negative, cells were pleiomorphic, bluntly rod, oval, or coccus shaped show (figure 4.3), which agree with the previous study (Benson, 2001). Motility test was positive, and starch hydrolysis was also positive where *A. chroococcum* able to utilize starch as the sole carbon source (Jan, 2006). From the morphological and biochemical tests its concluded that the isolated strains was *A. chroococcum.*

5.2 Use of *Azotobacter chroococcum* as Biofertilizer

As shown from the result that the biofertilizer in all parameter is higher than control, which gives an indication that biofertilizer helped plant growth and been able to provide the plant with nitrogen, which is one of the most important nutrients for plant growth, as it promoted rapid growth, increased leaf size and quality, hastened crop maturity, and promoted fruit and seed development. Nitrogen is an integral part of chlorophyll manufacture through photosynthesis (Mikkelsen and Hartz, 2008). But the lack of other nutrients such as potassium and phosphorus make growth less than the growth of plants with a chemical fertilizer, where potassium is needed for the plant cell's metabolic processes and in influencing the action of enzymes, as well as in aiding the synthesis and translocation of carbohydrates. And root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality are the attributes associated with phosphorus nutrition (Ahmad *et al*, 2009, William, 2009).

The study took place in pots and used nutrient-poor soils which may reduce the work of bacteria, as well as the lack of any food for the plant only through nitrogen-fixing.

A. chroococcum uses carbon for its metabolism from simple or compound substances of carbonaceous materials in soil. Besides carbon, *A. chroococcum* also requires calcium for nitrogen fixation. Similarly, growth of *A. chroococcum* is required to have presence of organic nitrogen, micronutrients and salts in order to enhance the nitrogen fixing ability of *A. chroococcum* (Gül, 2003). Although the free-living *A. chroococcum* are beneficial nitrogen-fixers, their contri-bution to nitrogen enrichment of the soil is limited due to the fact that they would rather utilize NH_3 in soil than fix nitrogen. In other words, if ammonia is present in the soil, nitrogen fixation by these organisms is suppressed. Therefore, the addition of chemical fertilizers or organic adversely affect the performance of bacteria and thus the effect of bacteria alone is stronger than the effects when mixed with chemical fertilizers and organic (Benson, 2001).

Compost + biofertilizer are less than or equal to biofertilizer. Adding compost to the soil causes the bacteria to move to the analysis of these compost, therefore, the bacteria consume the nitrogen chain to itself to grow and multiply, and after the end of this stage bacteria begins in the analysis of compost and nitrogen production, at this time the plant may be beyond the stage of formation of vegetative growth. Thus, the addition of organic fertilizers with bacteria does not give a significant result compared to the biofertilizers where the decomposition of compost takes a long time to start supplying the plant nutrients.

The presence of bacteria with chemical fertilizer leads to the presence of two inhibition factors to bacteria, the first is the high amount of nitrates and secondly, the acidic environment due to the presence of chemical fertilizer. Through the results, we find that the use of *A. chroococcum* alone had a positive effect on the growth parameters of cucumber. The bacterial inoculants caused effective increased in growth parameters such as number and weight of yield, root and shoot length, wet and dry weight of root and shoot, N% of cucumber. The outcomes of this study showed that *A. chroococcum* play role as biofertilizer where it's clear that the use of *A. chroococcum* affect the growth of cucumber . Biofertilizer (two dose) don’t affect the growth of cucumber as biofertilizer (one dose) it may be due to the competition of bacteria.

5.2.1 Lengths of Cucumber

Inoculation with *A. chroococcum* promoted shoot length when compared to control. The inoculated plants, both root and shoot length more than control as shown at figure (4.7- 4.8). Where root elongation is associated with the production of IAA in early stages. The IAA content was increased in inoculated plants as compared to control and so increased root length, shoot length due to bacterial phytohormones. Also the lack of essential nutrient cause the elongation of roots to obtain nutrient (Hamid *et al* 2008, Hassan, 2009). This results are in concordance with most similar previous studies (Dhamangaonkar *et al*; 2009**;** Mahato *et al*; 2009).

5.2.2 Dry Weights of Cucumber

The growth of roots and shoots were increased in the presence of *A. chroococcum* as biofertilizer (Figure 4.9- 4.11). The addition of bacteria to the soil affects the increase in vegetative propagation as the bacteria are fixing nitrogen, which is an important factor in the stages of plant growth, especially the early stages where the stem, root and leaves grow in these stages. The bacteria provided the right amount of nitrogen, the plant grew very well during the initial stages and continued to grow, but lack of the other nutrients, which are very important for plant, cause growth weaker than the chemical. But this growth in the presence of nitrogen only is an excellent and clear.

Plant growth has declined in the final stages, especially the growth of the stem where there was a weakness in the stem as a result of lack of other nutrients, but dry weight of the stem was higher than the control, where stalk and stem strength, crop maturity and production, are the attributes associated with phosphorus nutrition. This results are in concordance with most similar previous studies (Bagyaraj *et al*; 1978, Abd El-Gawad *et al* 2006; Sharma *et al,* 2007, Rawia *et al*; 2009; Selvakumar *et al*; 2009).

5.2.3 Wet Weights of Cucumber

The wet weight of root and shoot of cucumber were high in the presence of *A. chroococcum,* where weight of wet root and wet shoot were in biofertilizer higher than control (Figure 4.12, 4.13). The whole wet weight is not accurate as the amount of water varies from one plant to another depending on the irrigation of these plants. This results are in concordance with most similar previous studies (Abd El-Gawad et al 2006; Selvakumar *et al*, 2009; Dhamangaonkar *et al*, 2009).

5.2.4 Nitrogen Percentage

The result of our study showed that there were significantly role of *A. chroococcum* as biofertilizer were it affect the growth through N-fixation, and it give high nitrogen percentage at shoot and root see figure (4.17, 4.18) . This results are in concordance with most similar previous studies (Qureshi *et al*; 2009).

5.2.5 The Number and Weight of the Last three Collections

Only the last three collections were weighed and this was not enough to compare the treatments and the efficiency of bacteria or chemical fertilizer or organic fertilizer to increase the production of plant. It was observed that the production of plants that inoculated with bacteria produce cucumber crop better than control. Of course the presence of nitrogen that was fixed with bacteria increased the vegetative growth more than increased production. This results are in concordance with most similar previous studies (Abd El-Gawad *et al* 2006, Milani *et al*, 2007, Rawia, 2009, Mirzakhani, 2009). Application of biofertilizers is an acceptable approach for higher yield with good quality and safe for human consumption.

In general it appears that, as expected, application of biofertilizers improved yield and other plant criteria; this has also been reported elsewhere (Tabrizi *et al* 2008). From the results of the experiment it is clear that biofertilizer shows better results as compare to that of the control. The main advantage of biofertilizer is that it does not pollute the soil and also does not show any negative effect to environment and human health. Chemical fertilizers were better than the biofertilizer and that due to the absence of other nutrients in plant inoculated with bacteria**.** And this can be overcome either by adding chemical fertilizers containing nitrogen only for plants which are chemical treated or add other nutrients such as potassium and phosphorus to plant inoculated with bacteria. Finally obtaining less amount of healthy products with less environmental disturbances is preffered over obtaining higher amount of non-healthy prouducts with more environmental disturbances.

Chapter 6
Conclusion & Recommendations

6.1 Conclusion

The present study investigated the influence of N-fixing bacteria *A.chroococcum*, isolated from the soil on the growth and yield of cucumber.

Result from the present study indicated that yield and growth of cucumber, have been affected by the inoculation with *A. chroococcum*, because these biofertilizers can fix the atmospheric nitrogen in soil. Seed inoculated with *A.chroococcum* increased yield and growth about 5 - 30%.

1- In most parameters, the biofertilizer were higher than control and nearly equal or sometimes higher than compost, 20% chemical + biofertilizer and organic + biofertilizer .
2- A high yield of cucumber was obtained in the presence of *A.chroococcum* alone when compared to control yield.
3- A high growth of root and shoot was obtained in the presence of *A. chroococcum* alone when compared to control yield.
4- Higher dry and wet root and shoot were obtained in the presence of *A. chroococcum* alone compared to control yield.
5- The length was higher in shoot and root of plant inoculated with *A.chroococcum* alone or compared to control yield.
6- The N% of shoot and root were high in the plant inoculated with *A. chroococcum* alone when compared to control yield.
7- The chemically fertilized plant showed the best growth in all cases.

6.2 Recommendations

1- The experiment may be repeated using another bacteria or using mixture of different bacteria such as N-fixing bacteria, Potassium Solubilizing Bacteria, phosphate Solubilizing Bacteria, or another N -fixing microorganism.
2- Using a wide range of plants which are important and consume large amount of chemical fertilizers.
3- Inoculation of bacteria by different preparation, such as immobilization.
4- Cultivation of the plant in the field instead of the pots, to provide an appropriate environment to the bacteria.
5- The experiment may be repeated without mixing chemical fertilizer and compost to bacteria but may adding some nutrients to the soil.
6- Using chemical fertilizer of nitrogen content only to compare its effect with the bacterial impact.

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Appendix

Organic fertilizer (shaham)

Shaham content:

N ……(2-3%),

P_2O_5 …….....(2-3%),

K2O……(2-3%),

Organic matter …….(55-60%),

Humic acids..... 18%

Moisture ……. …12-8%

Ca…… ..9 – 8%

Mg……. 1 – 0.9%

Fe…….1 – 0.7%

Zn…….. 0.017%

Chemical fertilizer (14-14-14)

Total Nitrogen (N)* 14.0%

Nitrate Nitrogen (N) 5.80%

Ammoniacal Nitrogen (N) 8.20%

Available Phosphate (P2O5)* 14.0%

Soluble Potash (K2O)* 14.0%

Chlorine not more than 1.0%