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Soil-Plant Interface, Carbon Sequestration and the Eco physiological Growth of Salsola Imbricata and Zygophyllum Mandaville Using Locally Grown Rhizosphere and Endophytic Bacteria

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SOIL-PLANT INTERFACE, CARBON SEQUESTRATION AND THE ECO-PHYSIOLOGICAL GROWTH OF *SALSOLA IMBRICATA* AND *ZYGOPHYLLUM MANDAVILLEI* USING LOCALLY GROWN RHIZOSPHERE AND ENDOPHYTIC BACTERIA

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Environmental Sciences

Under the Supervision of Professor Taoufik Ksiksi

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Declaration of Original Work

I, Nour ElHouda Debouza, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Soil-Plant Interface, Carbon Sequestration and the Eco-Physiological Growth of Salsola imbricata and Zygophyllum mandavillei Using Locally Grown Rhizosphere and Endophytic Bacteria*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Prof. Taoufik Ksiksi, in the College of Science at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Copy 7 of 7

Abstract

Salsola imbricata and *Zygophyllum mandavillei* are halophytic plants available widely in the UAE. These two species have important environmental services such as sand dune fixation which will potentially improve plant cover and help tackle the problem of desertification. The species were selected due to their availability and role in the desert environment of the UAE. Plants are also constantly involved in interactions with a wide range of bacteria in the soil. These plant-associated bacteria colonize the rhizosphere (rhizobacteria), and the internal plant tissues (bacterial endophytes). Endophytic bacteria are those capable of colonizing live internal plant tissues which can be isolated from surface-disinfested plant material, and that do not visibly harm the host plant. In the present thesis, *Salsola imbricata* and *Zygophyllum mandavillei* were cultivated with or without the incorporation of plant growth promoting soil and endophytic bacteria that were obtained from *Salsola imbricata* and *Zygophyllum mandavillei* roots and soil. These plant growth promoting bacteria were selected based on their abilities to produce plant growth regulators such as auxins, polyamines and in addition to their abilities to fix nitrogen and to solubilize phosphorus. The aim of the present work was to examine if these bacteria can promote *Salsola imbricata* and *Zygophyllum mandavillei* growth without using large quantities of water, and the plants' carbon sequestration potentials. The plant species physiological growth pattern was closely monitored. The inoculation was effective in some growth parameters in both species after the application of treatment. Inoculated *Salsola imbricata* plants had larger root weight than control plants after four months of treatment, 0.50 g and 0.23 g respectively. Results from the current study state that inoculated soils had more activity than the control ones even after four months of

inoculation, meaning that the inoculation was successful and effective. However, significant changes in all physiological and morphological parameters were not observed. The parameters improved by inoculation include green shoot weight, root length, dry root and shoot weight, and chlorophyll content.

Keywords: Halophytes, plant growth promoting bacteria (PGPB), water stress, soil inoculation.

Title and Abstract (in Arabic)

ارتباط التربة و النبات ، حبس الكربون ، و النمو الفيسيو إيكولوجي لنباتات السالسولا إمبريكاتا و زيغوفلويم ماندياڤيلي باستخدام بكتيريا محلية.

الملخص

Salsola imbricate (الهرم) و *Zygophyllum mandavillei* هي نباتات ملحية متوفرة بكثرة في دولة الإمارات العربية المتحدة. هذه النباتات لديها خصائص مفيدة تجاه البيئة مثل تثبيت الكثبان الرملية و هذا بالمقابل سوف يحسن من المساحات الخضراء و يساعد في الحد من ظاهرة التصحر. لقد تم اختيار الفصيلتين بسبب وفرة هذه النباتات و دورها في الطبيعة الصحراوية لدولة الإمارات. تتعرض النباتات بشكل دائم إلى تفاعلات عديدة مع الكائنات المجهرية المتوفرة في التربة. البكتيربا المتعلقة بالنبات متواجدة بكثرة في التربة المحاطة بالجذور و مع الأنسجة الداخلية للنبات. ويمكن عزل هذه البكتيريا من عينات التربة و من الأسطح المعقمة لجذور النبات. في هذه الدراسة ، لقد تم إنبات الفصيلتين المذكورتين أعلاه باستخدام و بدون استخدام باكتيريا نافعة مستخلصة من جذورها و تربتها بهدف معرفة أثر هذه البكتيريا على نسبة النمو. لقد تم اختيار البكتيريا النافعة بناءً على قدرتها على إنتاج منظمات نمو النبات مثل هورمون الأوكسين و أنزيم (ACC) ، و كذلك على قدرتها على تثبيت النيتروجين وإذابة الفوسفور في التربة. الهدف من هذه الدراسة هو اختبار مدى قدرة هذه البكتيريا النافعة على تحسين نمو النبات باستخدام كميات أقل من الماء ، و كذلك اختبار جاهيزيتها في حبس الكربون من الجو. لقد تمت مراقبة أنماط النمو الفيسيولوجي للنباتات عن قرب خلال فترة التجربة و تم استنتاج أن التلقيح بالبكتيريا كان له أثر أيجابي على خصائص النمو لكلا الفصيلتين. وجد أن نباتات الهرم الملقحة بالبكتيريا كان لديها أوزان جذور أعلى (0.50 g)من التي لم تتعرض للتلقيح بالبكتيريا (0.23 g) بعد أربعة أشهر من تطبيق المعالجة. تظهر تنائج من هذه الدراسة أن التربة الملقحة بالبكتيريا كان لديها نشاط مايكروبي أكثر من التربة الغير ملقحة بالبكتيريا، مما يعني

أن عملية التلقيح بالبكتيريا ناجحة و فعالة. بالرغم من ذلك، لم يتم ملاحظة تغيرات معنوية في جميع خصائص نمو النبات. الخصائص التي تحسنت بعد تطبيق تلقيح البكتيريا تشمل وزن الساق ، وزن الجذور ، محتوى الكلوروفيل ، وزن الساق و الجذور الجاف.

مفاهيم البحث الرئيسية: النباتات الملحية، محسنات نمو النبات، الجفاف، تلقيح التربة بالبكتيريا

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Dedication

To my beloved parents, Mohemmed and El-Zohra Debouza

Table of Contents

List of Tables

List of Figures

List of Abbreviations

CC	Chlorophyll Content
PGPB	Plant Growth Promoting Bacteria
PR	Photosynthetic Rate
WS	Water Stress

Chapter 1: Introduction

1.1 Importance of plants

Plants are a vital part of the surviving of most species. They are used in producing food for human consumption, animal feed, or as elements for beautifying streets and lands. For each purpose, specific species are best to be used.

Great pressure is being placed on arable lands as the human population continues to grow. This pressure is a result of the constant demand for crops and forage to produce food resources for people. The balance of the ecosystem can easily be damaged if unplanned farm construction was performed. According to the World Food Program, one in nine people suffer from hunger (Ravallion, 2017). In order to contribute to the tackling of this issue of world hunger, new methods for plant production need to be considered and developed. It is also important to note the significance of plants in resolving some of the environmental problems such as desertification. Arid and semiarid environments provide habitat to more than one billion humans and they cove over 40% of the land surface on earth (Veron *et al*., 2006). People who live in these areas depend mainly on the efficient use of natural resources. However, it is widely known that these lands are at risk of desertification. Desertification is land degradation arid, semiarid, and dry sub-humid areas resulting from various factors such as climate changes and human activities, and this pose a serious threat to the environment and human welfare (Veron *et al*., 2006). To combat desertification plants play an ecological role in minimizing its negative consequences.

Plant species, especially in arid environmental like the UAE, have an array of uses, such as landscaping and forage production, both of which improve soil carbon sequestration. Forages can be defined as fibrous plant materials that are harvested or best utilized from other plants to feed farm animals. There are many species that have the potential to be classified as forage, and the most readily available ones are grasses such as Orchardgrass (*Dactylis)* and legumes such as alfalfa (*Medicago sativa*) (Capstaff & Miller, 2018). Landscape plants are the plans used to enhance the appearance of any type of land. Depending on the climate and available resources, the common species of landscaping plants vary from one county to another. In the UAE for example, Date palms (*Phoenix dactylifera)* and *Arabian Almond (Prunus arabica*) are often planted (Almehdi *et al*., 2005).

1.2 *Salsola imbricata* and *Zygophyllum mandavillei*

Salsola imbricata and *Zygophyllum mandavillei* are native halophytic plants widely available in the UAE with several beneficial properties (Jongbloed *et al*., 2003).

Plants that belong to the genus *Salsola* are frequently found in arid and semiarid regions of the planet. They typically grow on flat, dry and somewhat saline soils with other species that live in salt swamps**.** *S. imbricata* is a shrub that widely grows in Egypt and used as camel food (Osman *et al*., 2016). Moreover, Bushman women in Namibia and the Republic of South Africa use aqueous extracts of *Salsola* species in traditional medicine and as an oral contraceptive (Amann & Smith, 2005). Another species, *Salsola baryosma* is used in the Middle East against inflammations and as a diuretic agent. It has also been reported that some *Salsola* species have central nervous system depressant activity and antioxidant properties (Hamed *et al*.,

2011). *S. imbricata* is found along both costs of the UAE, and it grows on disturbed saline habitats. It can either be annual or perennial and the stems are typically straight (from 30 to 80 cm). The leaves change depending on the season and the flowers are yellow with a diameter of 0.5 to 1 cm. The small leaves are known to have unpleasant smell when crushed. *S. imbricata* is one of the first plants to colonize costal landfills (Jongbloed *et al*., 2003)**.**

The genus *Zygophyllum* denotes the biggest genus in the family Zygophyllaceae. *Zygophyllum aegyptium* is a perennial, woody bush with succulent leaves that remain green. This species is circulated in the Mediterranean area of Tunisia, Egypt, and Cyprus. Many species of the genus *Zygophyllum* have been used in removal of stiff spots on the skin, skin cleansing, in addition to illnesses, such as asthma, hypertension, rheumatism, and gout (Zaki *et al*., 2016). *Z. mandavillei* are perennial plants with brunched stems that reach up to 80 cm with succulent leaves that are cylindrical (0.3 x 0.5-1.5 cm). They are common on sand plains of Abu Dhabi emirate. The flowers are solitary with 0.5 cm across and 5 white petals half hidden in hooded green sepals on a 0.3 cm long stalk (Jongbloed *et al*., 2003). Apart from their medical uses mentioned above, the two species have other benefits that specifically help the environment (Abideen *et al.*, 2011).

Salsola imbricata and *Zygophyllum mandavillei* along with other halophytes have been proven to have the ability to fix sand dunes in the desert (El-Keblawy, 2013; Lecoustre, 2011). By stabilizing the sand dunes, desertification rates will decrease as less sand will be transported to non-arid lands. Moreover, these species can tolerate high salt concentrations and saline water can be used for irrigation (Abideen *et al.*, 2011). The water use efficiency is also high in these two species,

meaning that they don't require large amounts of water for irrigation. Since these two species produce flowers after they are mature, floral diversity is encouraged (Jongbloed *et al*., 2003). Such desert plants provide shade to the land and the increase of their numbers lowers the chances of land degradation as well as erosion rates (Reynolds *et al*., 2007)**.** For plants to grow and flourish properly, there are a number of elements that impact their performance and photosynthesis rate is an essential element (Verma *et al*., 2013).

1.3 Photosynthetic rate and chlorophyll content

Photosynthesis is the most essential physiological process in all green plants. Inside the chloroplasts of advanced algae and plants, photosynthesis transforms light into biological energy, utilizing the abundant atmospheric carbon dioxide and water into biologically valuable molecules (Berry *et al*., 2013). The rate of photosynthesis occurrence differs from one species to another and depends on many factors such as environmental conditions and stress (Ashraf & Harris, 2013)

The chlorophylls (Chl a) and (Chl b) are vital pigments for the translation of light energy to stored chemical energy in plants. The quantity of solar ray captivated by a leaf is a function of the photosynthetic pigment content; therefore, chlorophyll content directly determines primary production and photosynthetic potential. Also, Chl provides an indirect approximation of the nutrient content in the plant because a lot of leaf nitrogen is combined in chlorophyll. In addition, there is a close relationship with leaf chlorophyll content and plant stress and senescence. By tradition, spectrophotometric determination in solution and leaf extraction with organic solvents is mandatory for pigment examination with wet chemical method (Gitelson *et al*., 2003). Photosynthetic pigments play a role in photosynthesis

because they absorb light and transfer the energy to the chlorophyll molecules of reaction locations. Photosynthetic pigment content drops from ideal conditions in many plant species during drought. Plants decrease chlorophyll content in dry conditions because it is a tool for the avoidance of photosynthetic harm by permitting less light to be captivated (Viljevac, Dugalić, *et al*., 2013).

1.4 Types of stress

Plants constantly face a wide range of environmental stresses which creates a restriction to agricultural efficiency. The environmental stresses faced by plants can be classified as abiotic stress and biotic stress. Examples of abiotic stresses include drought, flood, and salinity, extremes in temperature, radiation and heavy metals. Abiotic stress is a leading factor that causes the loss of major crop plants globally. This situation will be more harsh due to increasing desertification of world's lack of water resources, increasing salinization of soil and water, and environmental pollution (Verma *et al.*, 2013). Biotic stress, on the other hand, includes attack by numerous living pathogens such as fungi, bacteria, nematodes, oomycetes, and herbivores. Infections created due to these pathogens are responsible for major yield loss worldwide. Because plants are sessile, they can not escape from these environmental stresses. To combat these threats, plants have developed various mechanisms for getting adapted to such conditions for survival (M. Ashraf & Harris, 2013).

Plants have the ability to feel the outside stress environment, become stimulated and then produce suitable cellular reactions. These cellular reactions work by sending the stimuli from sensors that are positioned on the cell external or cytoplasm to the transcriptional mechanism which is located in the nucleus with the

aid of many signal transduction trails (Viljevac *et al*., 2013). This results in a degree of difference in transcriptional alterations, making the plant tolerant to the stress. The signaling trails play an essential part and act as a joining link between sensing the stress environment and generating an appropriate physiological and biochemical response. As the mechanism of photosynthesis includes various steps, including photosystems and photosynthetic pigments, CO_2 reduction pathways and the electron transport system, any misplacement at any level initiated by a stress may decrease the overall photosynthetic ability of a green plant (Verma *et al*., 2013)

Various stressful environments have been stated to decrease the contents of photosynthetic pigments. For instance, salt stress can break down chlorophyll. This effect is linked to amplified level of the toxic cation Na+. Even though salt stress decreases the chlorophyll content, the degree of the decrease is dependent on salt tolerance of plant species. It is commonly known that in salt tolerant species (like *Juniperus virginiana),* chlorophyll content increases while it decreases in non-salt tolerant species (like *Pisum sativum*) under saline conditions (Ashraf & Harris, 2013). Heat is another form of stress that impacts plants and results in membrane disruption, particularly in thylakoid membranes. This thereby prevents the activities done by membrane-linked electron carriers and enzymes, ultimately resulting in a reduced frequency of photosynthesis. As in salinity stress, drought stress results in not only a considerable damage to photosynthetic pigments, but it also leads to the decline of thylakoid membranes (Ashraf & Harris, 2013). The root systems can also be greatly impacted by stress factors (Kramer & Boyer, 1995).

1.5 Morphology of roots

The roots are a vital part in all plants, for they provide a number of advantages. The purposes of roots include the absorption of water and mineral nutrients from the soil or any growing medium, anchorage, synthesis of various necessary compounds like growth regulators, and the storage of food in root crops like in sugar beet (*Beta vulgaris)* and cassava (*Manihot esculenta)* (Kramer & Boyer, 1995). Previous researches in environments that were water limited don't suspect that sizes and shapes of root systems differ among plants from arid to humid systems. For instance, plants are predicted to have larger root-shoot ratios in drier than in more humid environments. Also, maximum root depth spreads could still be larger in more humid environments because they naturally grow bigger there. This topic of large and small plant root growth is important because it helps with understanding ecological processes at different scales (Schenk & Jackson, 2002). Growth parameters are used to measure the development of the different parts of plants and the overall growth (Hunt, 1978).

1.6 Growth parameters

Growth parameters are referred to the set of quantitative methods that describe and predict the performances of whole plant systems grown under natural, controlled or semi natural conditions. Plant growth analysis provides a comprehensive approach to understanding plant function and form. Primary data such as weights, volume, areas and contents of the plant is sufficient to be used in investigating functions within plants (Hunt, 1978). Examples of plant growth parameters in crops and herbs include leaf area, root growth, height and biomass. These parameters are the origin for the foundation of many ecological and biological

models, including those for crop growth prediction, yield loss, and crop-weed competition. Since these parameters are suggestive of the plant's physiological state, they can also offer beginnings for site management approaches and decisions regarding fertilization, irrigation, and pest management. To assess parameters effectively, it is vital for any method to be dependable and accurate. Moreover, for precision agriculture, it should also be non-damaging and applicable on a large scale (Lati *et al*., 2013). The root and shoot systems are closely related.

1.8 Root and shoot ratio

Shoot growth is highly sensitive to stress conditions and especially to water stress (dry soil). Root growth is typically less inhibited than shoot growth in plants growing in drying soil; therefore it’s important to keep a sufficient plant water supply. A significant characteristic of the root system response is the ability of some roots to last elongation at water potentials that are small enough to entirely prevent shoot growth. For instance, this happens in nodal roots of maize that have to enter in the dry surface soil, and in primary roots of a number of other species that helps sprout formation in dry environments by confirming a supply of water before shoot development (Sharp, 2002). A relatively new method on increased plant production is the use of some microbial species (Bashan & Holguin, 1998).

1.9 Soil and plant interface: Rhizosphere

Plants are constantly involved in interactions with a wide range of bacteria. These plant-associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes), and the internal plant tissues (endophytes) (Glick *et al.*, 2007). Plant beneficial soil and rhizosphere bacteria are of two general types: those

that are free-living in the soil; which are often found near the rhizosphere, or even within the roots of plants as endophytes. In addition, there are types that form a symbiotic relationship, which involves formation of nodules on host plant roots such as root nodule bacteria (Dinesh *et al.,* 2015; Glick *et al.,* 2007). Beneficial free-living soil and rhizosphere bacteria are often referred to as plant growth-promoting rhizobacteria (PGPR) or plant growth-promoting bacteria (PGPB) and are found in a close association with the root surfaces of many different plants (Lucy *et al*., 2004).

However, to be inclusive of the many different types of bacteria that facilitate plant growth, the term plant PGPB, is preferred (Bashan & Holguin, 1998). The use of PGPR for the benefits of agriculture is gaining worldwide importance and acceptance and appears to be the trend for the future (Pitman & Läuchli, 2002). PGPR have economic and environmental benefits, which include high income from high yields, reduced fertilizers cost, reduced emission of the greenhouse gas, N_2O. They affect soil conditions, nutrient availability, tree growth and yields (Aslantaş *et al.,* 2007). PGPR have high diversity, they are environmentally friendly microorganisms. PGPR inoculation proven a promising agricultural approach that helps in soil restoration, crop production, nutrient recycling, growth promotion and disease control (Laslo *et al.,* 2012). These beneficial, free-living bacteria colonize roots, enhance yield, enhance emergence, and stimulate growth (Pitman & Läuchli, 2002).

PGPR benefits the sustainable agriculture system as it enhances the biological quality of soils through enhanced microbial and enzymes activity (Dinesh *et al.,* 2015). It is used in combination with fertilizers and manures to improve crops yields. It has positive effect on cereals, vegetables, flowers and spices (Dinesh *et al.,* 2015).

PGPR bacteria may improve plant growth or yield by direct or indirect mechanisms (Patel *et al.,* 2012). Direct mechanisms may involve the production of plant growth regulators such as auxins, gibberellins, cytokinins, or ethylene synthesis inhibitors which act directly on the plant itself and affect growth, synthesis of siderophores sequestering iron from the soil for plant use, the fixation of atmospheric nitrogen that can be used by the plant, and solubilization of minerals including phosphorus (Glick, 1995). Indirect mechanisms of growth promotion include the production of iron-sequestering siderophores (preventing iron acquisition by harmful microorganisms) or compounds that may have antifungal or antimicrobial properties, and thus serve to protect plants from soil phytopathogens (Glick, 1995). A particular bacterium may affect plant growth and development using anyone, or more, of these mechanisms. Moreover, since many PGPR possess several characters that enable them to facilitate plant growth, a bacterium may utilize different characters at various times during the life cycle of the plant, and may vary considerably in its effectiveness depending upon the plant host and the soil composition (Glick *et al.,* 2007).

The presence of endophytic bacteria inside numerous plant tissues are very common phenomenon (Jalgaonwala *et al.,* 2011; Lodewyckx *et al.,* 2002). In addition, endophytic bacteria have been isolated from leaves, seeds, flowers, stems fruits, roots, and ovules of various plant species (Kobayashi & Palumbo , 2000). These endophytic bacteria belonging to over 20 genera have been isolated from a variety of plants (Hallmann *et al.*,1997; Kloeppe *et al*., 1999).

Such endophytic bacteria are indigenous to most plant species, colonizing the tissues systemically or locally and both intracellularly and intercellularly (Gyaneshwar *et al*., 2001; Omarjee *et al.,* 2004). Several recent studies have shown that the interaction between plants and some endophytic bacteria was related with

beneficial effects such as induction of systemic resistance to plant pathogens (Andreote *et al.,* 2010; Benhamou *et al.,* 2000), biological control of insects (Azevedo *et al*., 2000; Campos *et al.,* 2010). Biological control of plant-parasitic nematodes (Neher, 2010), plant growth promotion (Bacon & Hinton, 2002; Patel *et al.,* 2012) , nitrogen fixation (Rout & Chrzanowski, 2009), biological control of pathogenic bacteria (Mastretta *et al.,* 2009), biological control of pathogenic fungi (El-Tarabily, 2003), crop adaptation to stress environment such as drought and salinity (Grover *et al.,* 2011), and improvement of phytoremediation (Khan & Doty, 2011). However, many endophytic bacteria have not yet been found to exert any beneficial effects on the host plant (Sturz & Nowak, 2000).

Compared with rhizosphere colonizers, internal colonizers can provide extra benefits. Because the plant provides shelter and nutrients, endophytic bacteria can develop under less competitive conditions and protect the plant interior against plant pathogens and adverse environmental conditions. Endophytes offer the double benefits of being adapted to their hosts, and present at seedling development and rhizosphere initiation. These factors provide endophytes with a competitive ecological advantage compared to the resident soil microflora that are so often implicated in the failure of biological seed treatments (Patel *et al.,* 2012).

1.10 Endophytic bacteria

Endophytic bacteria have several attributes which make them attractive as potential plant growth promoters. They colonize and form associations within plant tissues without causing disease, are protected from variable environmental conditions and from competition for limited space and nutrients (Lodewyckx *et al.,* 2002). Compared with rhizosphere colonizers, internal colonizers can provide additional

benefits. Because the plant provides shelter and nutrients, endophytic bacteria can develop under less competitive conditions and shield the plant interior against plant pathogens and adverse environmental conditions. Endophytic bacteria offer two benefits of being adapted to their hosts, and present at seedling growth and rhizosphere origination. These factors equip endophytic bacteria with a competitive ecological benefit equated to the local soil microflora that are so often involved in the dysfunction of biological seed treatments (e.g. biocontrol agents and growth promotion modifications) (Lodewyckx *et al.,* 2002).

Microbial endophytes are defined as "bacteria or fungi, which for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease", or "those which can be extracted from inner plant parts or isolated from surface-disinfected tissues and that do not visibly harm the plant" (Hallmann *et al.*, 1997).

Endophytic bacteria colonize herbaceous and woody mono-and-dicotyledonous including terrestrial and aquatic plants. They are found in the cortical and vascular tissues of roots, stems, tubers, leaves, flowers, fruits, seeds and ovules of a wide range of agricultural, horticultural, and forest species including alfalfa, pea, soybean, pear, potato, sugar beet, citrus, rice, cotton, cherry, grasses, canola, tomato, pine, oak and elms. Bacterial genera most commonly isolated include: *Azospirillum, Azoarcus, Herbaspirillum, Flavobacterium, Pantoea, Pseudomonas, Bacillus, Burkholderia, Citrobacter, Klebsiella, Serratia, Corynebacterium, Curtobacterium, Enterobacter* and *Streptomyces* (Hallmann *et al.*, 1997).

Several recent studies have shown that the interaction between plants and some endophytic bacteria was associated with beneficial effects such as plant growth

promotion (Torres *et al.,* 2012) nitrogen fixation (Cocking, 2003), biological control of pathogenic fungi (El-Tarabily *et al.,* 2010) biological control of plant-parasitic nematodes (Siddiqui & Shaukat, 2003), biological control of insects (Downing *et al.,* 2000); induction of systemic resistance to plant pathogens (Benhamou *et al.,* 2000), improvement of phytoremediation (Lodewyckx *et al.,* 2002), and crop adaptation to stress environment (Nowak *et al.,* 1998).

Endophytic bacteria-plant interactions have a potential role in developing sustainable systems of crop production (Rosenblueth & Martínez-Romero, 2006).

Recent successes using endophytic bacteria as agricultural inoculants (Hallmann *et al.,* 1997) are encouraging and were shown to provide an effective method to increase productivity of field crops. Nowadays, there is at present great interest in the introduction and/or manipulation of endophytic bacteria to provide a consistent and effective increase in the productivity of crops. Bacteria that have beneficial effects on plant health are referred to as beneficial plant-associated bacteria, plant-growth-promoting bacteria, or plant-growth-promoting rhizobacteria (Kloeppe *et al.,* 1999). The use of these beneficial bacteria has a great promise in agricultural crop production (Glick *et al.,* 2007; Whipps, 2001).

Plant growth effects attributed to plant-growth-promoting bacteria that have included endophytic bacteria include growth and developmental promotion (Frommel *et al.,* 1993), growth stimulation indirectly through the suppression of deleterious microflora in the root zone through competition for nutrients, siderophores-mediated competition for iron (i.e., can solubilize and sequester iron from the soil and provide it to the plant), and antibiosis (Kloeppe *et al.,* 1999).

Growth stimulation can also be achieved through the direct production of plant growth regulators (PGRs) such as auxins, gibberellins and cytokinins which in

very low quantities enhance various stages of plant growth (Bastián *et al.*, 1998), indirect growth stimulation through the induction of phytohormone synthesis by the plant (El-Tarabily *et al.*, 2009), growth promotion through the enhanced availability of minerals especially phosphorus (Kloepper *et al.*, 1989), fixation of atmospheric nitrogen and supply it to plants (Reinhold-Hurek, 1998), production of low-molecular-mass compounds or enzymes that can modulate plant growth and development (Glick, 1995), and alteration of the plant susceptibility to frost damage (Xu *et al.*,1998).

A particular plant-growth-promoting bacterium may affect plant growth and development by using any one or more of these mechanisms (Glick *et al.*, 2007). It is probable that the same is true for endophytic bacteria as suggested by Lodewyckx *et al.* (2002).

Plant growth regulators, such as auxins, cytokinins and gibberellins, produced by some strains of endophytic bacteria such as *Pseudomonas*, *Enterobacter*, *Azotobacter*, and *Azospirillum*, may also be considered to be causal agents for plant growth promotion (Bashan & Holguin, 1997). *Azospirillum*, for instance, is generally regarded as being a rhizosphere bacterium that colonizes mainly the elongation and root hair zones of roots (Bashan & Holguin, 1994).

However, some *Azospirillum* strains can also be endophytic, being found within the roots of some Gramineae (Bashan & Holguin, 1994) . The observed plant growth promotion after inoculation of plant roots with *Azospirillum* is thought to be due to the production of auxins by the endophytic bacterium (Barbieri & Galli, 1993). The endophytic bacteria *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* have been shown to produce indole-3-acetic acid and gibberellins in chemically-defined culture media (Bastián *et al.*, 1998).

Another way in which plant-associated bacteria might influence plant growth has been discussed by Glick (Glick *et al.,* 1994). They demonstrated that many plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. This enzyme might be part of a mechanism used by certain bacteria to stimulate plant growth as suggested by Glick *et al*. (1994). This enzyme could modulate the level of ethylene in developing plants (Glick *et al.,* 1994). It is also well known that plants respond to a variety of different environmental stresses by synthesizing "stress" ethylene. A noteworthy part of the harm to plants from environmental stress, such as infection with fungal pathogens, may happen as a straight result of the response of the plant to the amplified level of stress ethylene. Because ACC deaminase may act to warrant that enlarged ethylene levels are dropped in a developing or stressed plant, it may improve the plant's suitability, and consequently can be considered to behave as a plant growth-promoting characteristic (Glick *et al.,* 1994).

Endophytic bacteria have been reported to promote and enhance growth of several plants, including potato (Sturz, 1995), lodge pole pine (*Pinus contorta*), (Bent & Chanway, 1998), rice (Hurek *et al.,* 1994; Prayitno *et al.,* 1999), oilseed rape and tomato (Nejad & Johnson, 2000), corn (Bacon & Hinton, 2002; Riggs *et al.*, 2001) , soybean (Bai *et al.*, 2002), beans (Bacon & Hinton, 2002), and cucumber (El-Tarabily *et al.,* 2009).

The endophytic bacteria *Bacillus polymyxa* and *Curtobacterium flaccumfaciens* increased root growth (branching and elongation) and shoot biomass of pines 9 weeks after inoculation (Bent & Chanway, 1998). Nejad and Johnson (2000) reported that the application of endophytic bacteria either singly or in

combination significantly improved seed germination, seedling length and plant growth of oilseed rape and tomato.

When these endophytic bacterial isolates were used for seed treatment, they also, significantly reduced disease symptoms caused by the vascular wilt pathogens *Verticillium dahliae* and *Fusarium oxysporum* f.sp. *lycopersici* (Nejad & A Johnson, 2000). The endophytic bacterium *Pantoea agglomerans* was reported by Riggs *et al.* (2001) to increase corn productivity. Four endophytic bacteria isolated from rice roots and identified as *Pseudomonas fluorescens* (S3), *Pseudomonas tolaasii* (S20), *Pseudomonas veronii* (S21), and *Sphingomonas trueperi* (S12) were shown to promote rice growth (Adhikari *et al.*, 2001).

Bai *et al.* (2002) isolated endophytic *Bacillus subtilis* and *B. thuringensis* from surface sterilized soybean root nodules. These isolates were found to increase soybean weight when plants were inoculated with these endophytic bacteria. Inoculation with the endophytic bacterium *Bacillus mojavensis* increased growth of corn and beans. There was a 70% average increase in root and shoot growth in endophyte inoculated plants compared to the non-inoculated control plants (Bacon & Hinton, 2002).

1.11 Measurements of microbial activity

Soil represents a medium or substrate in which numerous microorganisms live and bring about a great variety of processes (Waksman, 1952). Agar plate methods are commonly used for the estimation of total soil microflora using selective media for each particular group of microorganisms (Crawford *et al.,* 1993; Rothrock & Gottlieb, 1984). Assessment of microbial populations in soil can be difficult for several reasons. For example, microbial cells are commonly attached to surfaces

where they live side-by-side with other populations containing different physiological and morphological types (Alef *et al.*, 1988). Such groupings of organisms cannot be described quantitatively using techniques such as the dilution-plate or dilution-count methods, which underestimate both cell numbers and viable biomass (Domsch *et al.*, 1979). These population counts can be at best, only rough estimates, since the microflora is diverse and not all organisms can be cultured on laboratory media (Alexander, 1977).

In addition to direct counting, several other methods are available to determine general microbial activity in soil. These include chemical assays of microbial biomass by the determination of ergosterol (Seitz, 1979), hexosamine (Blanchette, 1978), and ATP (Oades & Jenkinson, 1979). The determination of hexosamine and ergosterol has been applied mainly to fungi. Chemical estimations of microbial biomass however, assume a relatively constant ratio between the estimated chemical component and the total cell biomass from which the component is taken. The estimations, therefore, can be used only as an index of biomass, not as an absolute estimation of it. In such studies, cells grown in pure culture and not those from natural habitats are used for standardizing the assays (Swisher & Carroll, 1980). Unfortunately, both ATP and ergosterol determinations require expensive equipment and experienced laboratory personnel.

Measurements of dehydrogenase activity (Skujiņš, 1973), uptake of ^{14}C-labelled glucose (Waid *et al*., 1971), and respirometry (Hubbard, 1973), are also used to determine the microbial metabolic activity. Such techniques do not discriminate between active and inactive cells such as spores or quiescent vegetative cells, and therefore may seriously underestimate total active biomass (Swisher & Carroll,

1980). In addition, most of these methods are labor and time-consuming in addition to the need for expensive equipment.

Swisher and Carroll (1980) developed a method, based on the hydrolysis of fluorescein diacetate (3', 6'-diacetylfluorescein) (FDA) to determine the amount of microbial activity in needle litter, soil and litter. Schnurer and Rosswall (1982); Chen *et al.* (1988*a*, *b*); Inbar *et al.* (1991); Boehm and Hoitink (1992) reported the use of FDA hydrolysis to determine total microbial activity in soil, potting mix and straw litter, respectively. FDA has been used routinely as a vital fluorescent stain for soil fungi (Soderstrom, 1977). FDA, a non-fluorescent substrate, is hydrolysed by various enzymes (such as proteases, lipases and esterases) of living cells and yields fluorescein (Rotman & Papermaster, 1966). Fluorescein remains in the cell causing intracellular fluorescence which can be visualized by fluorescence microscopy (Schnürer & Rosswall, 1982) and can also be quantified by fluorometery or spectrophotometry. Earlier studies have shown that activity of all fungi investigated (Soderstrom, 1977), most bacteria (Lundgren, 1981), and some protozoa and algae (Medzon & Brady, 1969) could be assayed with FDA hydrolytic activity. The FDA hydrolysis technique can be considered as simple, inexpensive, and an accurate reflection of the activity of most microbes (Schnürer & Rosswall, 1982).

Another simple, rapid and inexpensive method to determine total microbial activity, based on the ammonification of arginine, was developed by Alef and Kleiner (1986). Their results are highly reproducible and correlate well with respiratory activities. Ammonification is defined as ammonia liberation from nitrogenous compounds which are used as C or N sources (Alef & Kleiner, 1986).

The rise of atmospheric carbon dioxide had disturbed the balance of many elements of the environment including plants (Lal, 2004).

1.12 Plant carbon sequestration

There has been an extreme growth in the atmospheric content of carbon dioxide (CO_2) and other greenhouse gases (GHGs) since the industrial revolution took place. The atmospheric concentration of CO_2 has amplified from 280 ppmv in 1750 to 367 ppmv in 1999 and is presently still growing at the rate of 1.5 ppmv per year. Methane (CH_4) levels in the air has enlarged from about 700 to 1745 ppbv over the same phase and is still growing at the rate of 7 ppbv per year (Lal, 2004). Soil carbon sequestration is the method of moving carbon dioxide from the atmosphere into the soil over crop remains and other organic solids, and in a shape that is not directly emitted again to the air. This sequestering of carbon helps to balance off some emissions from fossil fuel burning and other carbon-releasing actions while improving soil quality and long term agricultural production. Soil carbon sequestration can be established by management organizations that introduce large amounts of biomass to the soil, conserve soil and water, improve soil structure, cause minimal soil disturbance, and improve soil fauna activity (Sundermeier & Reeder, 2005).

Plant root function as a medium for removal of atmospheric carbon into the soil in the form of compounds containing carbon, like organic acid, phenolic acid, amino acid, etc. Root lysis and root exudates donate noteworthy amounts of carbon left in sub surface soil. Apart from surface soil, these deposits have the ability for a bigger influence to long-term soil carbon sequestration due to relaxed oxidation. Carbon components impact agriculture by reducing microbial growth, pH, and

nutrient mobilization. The particular quantity of sequestration relies on climate, edaphic factors, land-management practices, and the total number and quality of plant and microbial levels. Studies on carbon allocation via roots will create a new idea that will permit better judgments on the precise use of fertilization, soil amelioration, and crop rotation. These methods deliver valuable tools for addressing many problems in both natural and agricultural soils. Carbon sequestration will positively play a role in decreasing atmospheric CO_2 concentration and will lessen drought, desertification, and salinity stress. It will be a feasible approach towards sustainable agriculture. Therefore, sequestered soil and plant carbon may be used for forestry, agriculture, and ultimately be a potential option to lessen global change (Kumar *et al*., 2006).

According to the previous literature, PGPR has been shown to improve different growth parameter (like root system and shoot length) as well as productivity in different crops. Plants that have the ability to grow faster will contribute to the enhancement of desert ecosystems more efficiently, and capture more carbon from the atmosphere. However, implications of PGPR on native desert halophytes with the purpose of serving environmental services have not been done before. Therefore, the present study was done on UAE native species *Salsola imbricata* and *Zygophyllum mandavillei* to assess the impact of PGPR on their growth and carbon sequestration ability, aiming to enhance the quality of desert ecosystems.

The aims of the present work are therefore to improve the growth and productivity of the two halophytic plants *Salsola imbricata* and *Zygophyllum mandavillei* using locally isolated rhizosphere and endophytic bacteria, to isolate and to assess the ability of plant growth promoting rhizosphere and endophytic bacteria

to promote plant growth and productivity under UAE environmental conditions and to compare the performances of *Salsola imbricata* and *Zygophyllum mandavillei* after the inoculation with or without the beneficial plant growth promoting bacteria, to determine if carbon sequestration of soil is impacted with the incorporation of these rhizosphere and endophytic bacteria and to closely explore the interface between the soil and plant as well as the eco-physiological growth of *Salsola imbricata* and *Zygophyllum mandavillei*. The overall aim was to provide alternative species to be used in landscape planting as well as sand dune fixing.

Chapter 2: Materials and Methods

This chapter will include full details on the methodology and materials used in the experiments of this study. The methods were divided into three main sections: 2.1) Plant Growth Assessment part, 2.2) Agricultural Microbiology part and 2.3) Plant carbon sequestration part. The plant growth assessment part will discuss plant cultivation and maintenance methods and the agricultural microbiology part will explain how the final bacterial strains were obtained from both *Salsola imbricata* and *Zygophyllum mandavillei* and inoculation methods. Finally, plant carbon sequestration assessment will be discussed closely. The setup of the trial is outlined in Figure 1.

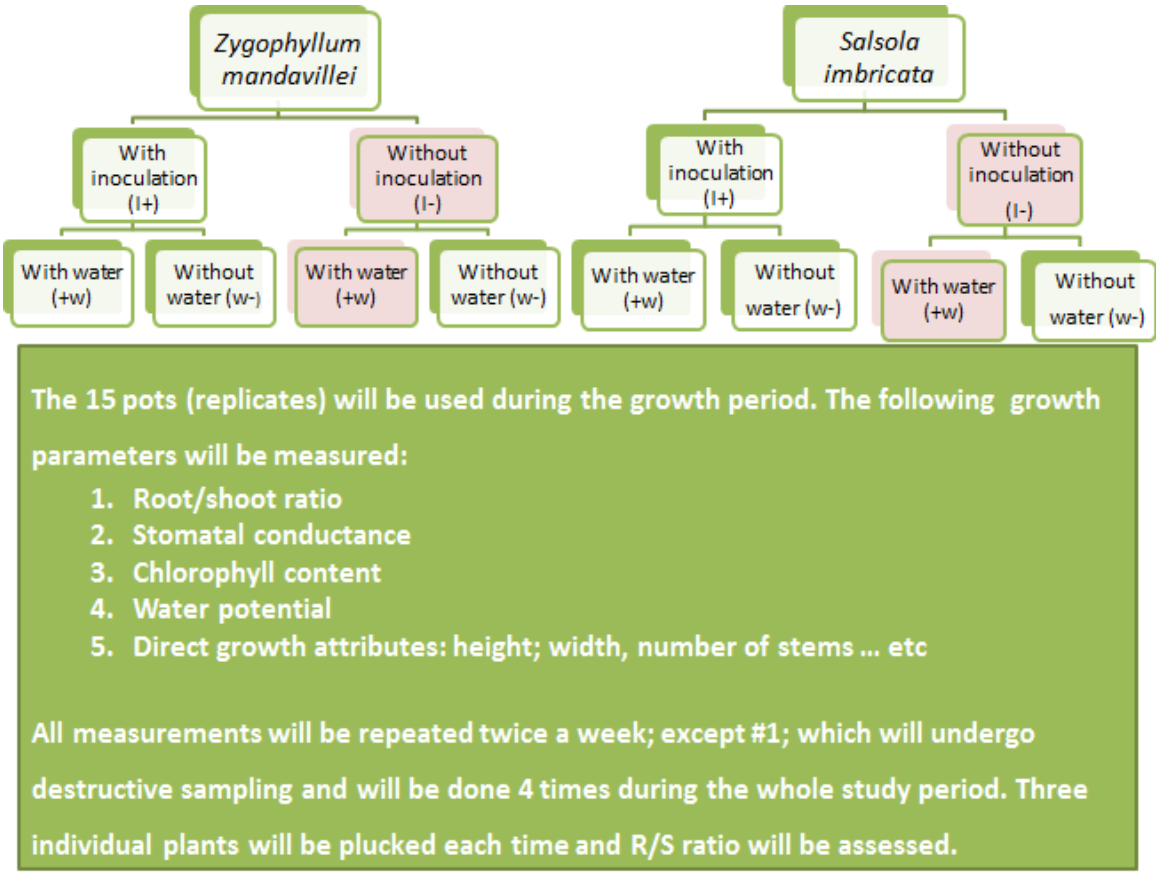

Figure 1: Graphical representation of the experimental design

2.1 Plant growth assessment

Seeds of *Salsola imbricata* and *Zygophyllum mandavillei* were collected from different desert areas around Al Ain city (24.1302° N, 55.8023° E). The seeds (4-8 seeds) were planted in 20 cm round pots with draining holes, and were watered as needed. Pots were kept inside green houses in Al Foa farm.

Once the plants were mature (5-6 months after cultivation), they were transferred from the farm and kept outside E3 lab in UAEU. The plants were watered twice a week; moisture content in the soil was kept at 30%. The inoculation was performed after the plant transfer adjustment period (2 weeks).

Initial growth measurements were done on randomly selected plants from both species. All measurements were repeated once a month during the entire experiment period (4 months). Growth measurements include: shoot/root ratios, plant height, chlorophyll content, and photosynthetic rate. Environmental conditions (temperature and soil moisture) were also measured. Growth parameters were monitored during that time using the following equipment:

a) ERAS miniPPM : photosynthetic rate
b) Hansatech (model CL-01) chlorophyll content meter: chlorophyll content
c) Extech MO750 Soil Moisture Meter: soil moisture

After the cultivation of plants, 2 randomly selected ones were used in the agricultural microbial activity part (Figure 2).

Figure 2: Samples of *Salsola imbricata* (left) and *Zygophyllum mandavillei* (right) with soil samples collected from areas around the roots

2.2 Microbiological assessment

2.2.1 Media

The following media were used in the present study. The composition of the media is listed in appendix 1.

1- Inorganic salt-starch agar (starch nitrate agar) (SNA) (Küster, 1959).

2- Glucose peptone broth (GPB) (di Menna, 1957).

3- Moeller's decarboxylase agar medium (MDAM) (Arena and Manca de Nadra, 2001).

4- Nutrient agar.

5- Nutrient broth.

6- Potato dextrose yeast extract agar.

2.2.2 Plant material and soil materials

Plant root and soil samples were obtained previously from plant growth assessment part. Seed of both *Salsola imbricata* and *Zygophyllum mandavillei* were collected from different desert areas in Al Ain city and then cultivated.

2.2.3 Enumeration of soil bacterial populations

For the isolation of bacteria and actinobacteria, mature plants of both species were gently plucked from pots and excess soil around the roots and small roots were collected. The bacterial populations of the freshly sampled rhizosphere soils were estimated using the soil dilution plate method (Johnson & Curl, 1972). Three 10 g replicates of each soil were dispensed into 100 mL of sterile 0.1% (w/v) agar (Gibco BRL, Paisley, Scotland) solution in de-ionized water containing 20 g glass beads (3 mm diameter). The soil suspension was shaken 50 times and then the ten-fold dilutions (10^{-1} - 10^{-5}) were made in sterile deionized water and 0.2 mL were spread with a sterile glass rod over the surface of nutrient agar medium containing the antifungal antibiotic cycloheximide (Sigma) (50 $\mu g mL^{-1}$) for the isolation of bacteria. Five plates were used per dilution. The plates were dried in a laminar flow cabinet for 30 mins and then incubated at 28 ± 2°C for 2-4 days and colony counts were carried out from day 2 onwards. Bacterial colonies were counted and were expressed as colony forming units (cfu) g dry^{-1} soil. All bacterial colonies were then transferred onto nutrient agar plates, and stored in 20% glycerol (cryoprotectant) at -20°C (Wellington, 1979).

For the isolation of actinobacteria, the rhizosphere soils were air dried for 4 days at 28°C to reduce the numbers of viable vegetative bacterial cells (Williams *et al*., 1972). Actinobacteria were then isolated and estimated using the soil dilution plate method (Johnson & Curl, 1972) on inorganic salt-starch agar (SNA) (Kuester, 1959) amended with the antifungal antibiotic cycloheximide (Sigma) (50 μgmL^{-1}) and nystatin (Sigma) (50 μgmL^{-1}) (Williams & Davies, 1967), which were added to the cooled (45°C) sterile molten agar immediately prior to pouring plates. Five plates were used per dilution, and the plates were incubated at 28 ± 2°C in the dark for 7 days. Actinobacteria colonies were counted on SNA medium and were expressed as cfu g dry^{-1} soil. All colonies were then transferred onto oatmeal agar plates supplemented with 0.1% yeast extract (OMYEA) (Williams, 1982) , and stored in 20% glycerol (cryoprotectant) at -20°C (Wellington, 1979). They were tentatively identified and grouped to the genus level on the basis of their standard morphological criteria and according to the absence or presence of aerial mycelium, distribution (aerial/substrate) and form of any spores present and stability or fragmentation of substrate mycelium (Cross, 1989).

2.2.4 Isolation of endophytic bacteria and endophytic actionbacteria from surface-disinfested *Salsola* and *Zygophyllum* roots

To isolate endophytic bacteria and endophytic actionbacteria, the roots cut from stems were rinsed in running tap water for 1 h to remove soil particles and surface contaminants and the fresh root weight recorded before further processing. Roots were soaked in sterile phosphate-buffered saline solution (PBS) (pH 7.0) for 10 min to equilibrate osmotic pressure and to prevent passive diffusion of sterilizing agents into the roots (Hallmann *et al.*, 1997). Roots were surface-disinfested by first

exposing them to propylene oxide vapor for 25 min (Hallmann *et al.*, 1997). They were then soaked in 70% ethyl alcohol for 4 min followed by immersion in 1.05% solution of commercial bleach and shaken by hand for 5 min. The surface-disinfested roots were then rinsed ten times (5 min each rinse) in sterile phosphate buffer (PB) (Hallmann *et al*., 1997).

To confirm that the surface disinfection process was successful and to verify that no biological contamination from the surface of the roots was transmitted into the root tissues during maceration, sterility checks were carried out for each sample to monitor the effectiveness of the disinfestation procedures. For these checks, root impressions were taken (Hallmann *et al*., 1997) and 0.2 ml from the final rinse was plated out on petri plates of nutrient agar (NA) , and potato dextrose yeast extract and potato dextrose yeast extract agar (PDA) (Difco) amended with 250 μgml^{-1} chloramphenicol (Sigma). The absence of bacterial, fungal including yeast growth after 6 days of incubation at 28°C for PDA and NA plates in the sterility checks was taken to confirm sterility and actinobacteria that were isolated were considered to be endophytic.

Roots were macerated in 100 ml of PB using a sterile mortar and pestle under aseptic conditions, and then shaken for 30 mins using a wrist-action shaker. The slurry was filtered through sterile filter papers, and the filtrate was serially diluted (10^{-1} - 10^{-5}) in PB (Hallmann *et al.*, 1997). Aliquots (0.2 ml) were spread with a sterile glass rod over the surface of nutrient agar amended with cycloheximide (Sigma) (50 μgmL^{-1}) for the enumeration of the total endophytic bacterial populations. In addition, aliquots (0.2 ml) were spread with a sterile glass rod over the surface of (SNA) amended with cycloheximide (Sigma) (50 μgmL^{-1}) for the

enumeration of the total endophytic actinobacterial populations. All plates were dried in a laminar flow-cabinet for 20 mins before incubation at 28°C in the dark for 7 days. Four plates per dilution were made for each root sample. Population densities were expressed as $\log_{10}$ colony forming units (cfu) g^{-1} fresh root weight (Hallmann *et al.*, 1997).

All bacterial isolates were transferred onto nutrient agar plates and all actinobacterial isolates were transferred onto oatmeal agar plates supplemented with 0.1% yeast extract (OMYEA) (Williams, 1982). All cultures were stored in 20% glycerol (cryoprotectant) at -20°C (Wellington, 1979).

2.2.5 Qualitative determination of indole-3-acetic acid

The aim of this experiment was to screen all the isolates (48 bacteria from *Salsola imbricata* soil, 36 bacteria from *Salsola imbricata* root, 4 actinobacteria from *Salsola imbricata* soil, 21 bacteria from *Zygophyllum mandavillie* soil, 13 bacteria from *Zygophyllum mandavillie* roots, 2 actinobacteria from *Zygophyllum mandavillie* soil. total: 124 strains) for their ability to produce indole-3-acetic acid (IAA) in glucose peptone broth (GPB) (Di Menna, 1957) amended with L-Tryptophan (L-TRP) (Sigma). Erlenmeyer flasks (250 mL) each containing 100 mL of sterile GPB were amended with 3 mL of 5 % filter sterilized L-TRP (Millipore membranes, pore size 0.22 µm, Millipore Corporation, MA, USA) *(*Khalid *et al.*, 2004).

The flasks were inoculated with 2 mL of each of the isolate prepared from a 5-day-old shake GPB culture. The flasks were covered with aluminum foil and incubated on a shaker (Model G76, New Brunswick Scientific, Edison, NJ, USA) at 250 rpm at 28 ± 2°C in the dark for 7 days.

Non-inoculated flasks served as controls. After incubation, the suspension from each flask was centrifuged for 30 min at 12000X *g*. The supernatant was filtered through sterile Millipore membranes (pore size 0.22 µm) and collected in sterile tubes. The culture supernatants (3 mL) were pipetted into test tubes and 2 mL of Salkowski reagent (2 mL of 0.5 M Ferric chloride + 98 mL 35% percholeric acid) were added to it (Gordon & Weber, 1951). The tubes containing the mixture were left for 30 min for red color development. The intensity of the red color was determined visually

2.2.6 Qualitative determination of polyamines production

The aim of this experiment was to screen all the isolates (48 bacteria from *Salsola imbricata* soil, 36 bacteria from *Salsola imbricata* root, 4 actinobacteria from *Salsola imbricata* soil, 21 bacteria from *Zygophyllum mandavillei* soil, 13 bacteria from *Zygophyllum mandavillei* roots, 2 actinobacteria from *Zygophyllum mandavillei* soil. total: 124 strains) for their ability to produce arginine decarboxylase and to produce putrescine (Put) from its corresponding amino acid arginine in a Moeller's decarboxylase agar medium (MDAM) supplemented with 2 g L^{-1} of L-arginine (Sigma) (Arena and Manca de Nadra, 2001). Five-days-old isolates grown on OMYEA were streaked in triplicate on MDAM plates. The plates were incubated at 28 ± 2°C in the dark for 2 days. Growth of the decarboxylating isolates was detected by the presence of a dark red halo around and beneath the colonies, compared to the yellow medium without inoculation of any isolate (control).

2.2.7 Qualitative determination of phosphorus solubilization

The aim of this experiment was to screen all the isolates (48 bacteria from *Salsola imbricata* soil, 36 bacteria from *Salsola imbricata* root, 4 actinobacteria from *Salsola imbricata* soil, 21 bacteria from *Zygophyllum mandavillei* soil, 13 bacteria from *Zygophyllum mandavillei* roots, 2 actinobacteria from *Zygophyllum mandavillei* soil. total: 124 strains) for their ability to solubilize insoluble calcium phosphate using Pikovskaya agar medium (PVK) (Pikovskaya, 1948) amended with bromophenol blue (Sigma). Each isolate was streaked in duplicate in the center of a plate and the plates were incubated at 28 ± 2°C in the dark for 4 days. Clear zone diameters were measured (mm) and were used as an indicator of phosphate solubilization. Large diameters (>20 mm) represented high activity and smaller diameters represented low activity. Three independent replicate plates were used for each isolate. Solubilization of calcium-phosphate was assessed by measuring the diameters of the clear zones.

2.2.8 Estimation of the total microbial activity

2.2.8.1 Preparation of standard curve for fluorescein diacetate (FDA) technique

Standard curves were prepared as described by Chen *et al.* (1988*a*, *b*) by adding various amounts of FDA, ranging from 0 to 400 µg from the stock solution in duplicate, to 5 mL of phosphate buffer in screw cap-tubes. Test tubes were capped tightly and heated in boiling water for 60 min to hydrolyze FDA (Schnürer & Rosswall, 1982). The hydrolyzed FDA was then added to the Erlenmeyer flasks containing 5 g (dry weight) soil samples. Another 15 mL of buffer was used to wash the hydrolyzed FDA from the tubes into the samples. The flasks were next shaken 20 min on a rotary shaker at 25°C, after which 20 mL of acetone were added. The

samples were filtered and processed as described above for the samples, and the absorbance was measured at 490 nm.

2.2.8.2 FDA hydrolysis technique

The microbial activity of the freshly sampled rhizosphere soils was measured by fluorescein diacetate hydrolysis. The hydrolysis of FDA (Sigma Chemical Co., St Louis, Mo., USA) was measured by the method of Schnurer and Rosswall (1982). Briefly, 5 g of each soil were added to 20 mL of sterile 60 m*M* potassium phosphate buffer (8.7 g K_2HPO_4 and 1.3 g KH_2PO_4 in 1 L distilled water, pH 7.6) in 250 mL flasks. The FDA was dissolved in acetone and stored as a stock solution (2 $mgmL^{-1}$) at -20°C. The reaction was started by adding 0.2 mL of FDA (400 µg) from the stock solution to a buffer-soil mix. Each treatment consisted of four replicates and one blank to which no FDA was added. The reaction flasks were shaken (90 rpm) at 25°C for 20 min on a rotary shaker (New Brunswick Scientific). The reaction was then stopped by adding 20 mL acetone to all samples. Soil residues were removed from the mixture by centrifugation at 500 rpm for 10 min and filtered through a No. 1 Whatman filter paper (Whatman, Maidstone, England). The filtrate was collected in a test tube, covered with Parafilm and placed into an ice bath to reduce volatilization of the acetone. The concentration of fluorescein was determined by reading the optical density at 490 nm, using a Shimadzu UV-2101/3101 PC scanning spectrophotometer (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). This permitted the rapid handling of many samples, the concentrations of which were compared against a standard curve. The background absorbance was corrected for each treatment with the blank sample run under identical conditions but without the addition of FDA. The results were converted to µg hydrolyzed FDA g dry^{-1} soil.

2.2.9 Soil inoculation and irrigation methods

Peat moss was placed in autoclave safe bags and was autoclaved twice to eliminate any microbes. The autoclaved peat moss was then placed in sterilized containers and a bacterial solution was added. The bacterial solution consists of a mixture of 15 promising PGPB which were incubated for 7 days in 300 mL of NB each. The peat moss mixture was placed in closed laminar flow overnight to dry. Once the inoculated peat moss was dry, 50 g were added around the root area of randomly selected plants, and sandy soil was added to cover the inoculated peat moss (Figure 3).

Figure 3: Soil inoculation in *Zygophyllum mandavillei*

2.2.10 Irrigation

After soil water holding capacity was performed, the 15 most promising PGPB (1-15) were cultured in 300 mL NB and incubated at 27°C for 7 days. Once fully developed, equal amount of sterilized water (4.5L) was mixed with all 15 liquid cultures. Table 1 shows the exact quantity of irrigation liquids as well as the frequency. The soil water holding capacity was found to be 400 mL.

Table 1: Irrigation methods for all four testing groups

Group	**Irrigation material**	**Quantity**	**Irrigation frequency**
Control	Water	400 mL	Once a week
Inoculation	Microbial solution	400 mL	Once a week
Inoculation + water stress	Microbial solution	200 mL	Once in 2 weeks
Water stress	Water	200 mL	Once in 2 weeks

2.3 Plant carbon sequestration

After the experiment time had elapsed, the tested plants were oven dried for 3 days. The dried soil samples were then ground to fine powder. Using oven safe crucibles, the powdered plant samples were ignited at 400°C. Sample weight was recorded twice (oven dry weight and ignition weight) to meet the equation mentioned by (Allen, 1989) :

$$\text{Organic Carbon \%} \frac{\text{Oven dry weight-ignition weight}}{\text{Oven dry weight}} \times 100$$

2.4 Statistical analyses

SPSS was used to analyze the quantitative data collected during this experiment. Bar graphs were used to compare the means of different groups, and profile graphs were used for organic carbon content assessment.

In certain research investigations, a one way analysis of variance (ANOVA) is suitable in studies where there are more than three groups or conditions, and also used to determine if they vary considerably on the same result. ANOVA test can be used to identify if groups have an identical mean. As t-test is confined to the condition when there are two groups; ANOVA is best suited for studies with more groups involved. The ANOVA test follows basic identical rules of data being continuous as the t-test (Dytham, 2011). In order to decide if the differences among the means are statistically noticeable, ANOVA test was used on the data obtained from the plants. The P-value is to be compared to the significance level of 0.05. The null hypothesis is that the means in all groups are equal. The Alternate hypothesis is that not all means in all groups are equal. If the P-value is less than or equal to the

value of 0.05, the null hypothesis is rejected, and it can be determined that the means in the population can be different and not all of them are equal. If the P-value is greater than 0.05, it indicates that there isn't sufficient proof for the null hypothesis to be rejected and so any difference between the means will not be significant enough. In this experiment, α will be considered (0.1) to determine the significance level between groups.

Chapter 3: Results

3.1 Bacterial properties

3.1.1 Isolation and classification of bacterial colonies

A total of 124 different microorganisms were isolated from the rhizosphere and roots of *Salsola imbricata* and *Zygophyllum mandavillei* (Figure 4). They were given serial numbers for better data handling and were primarily characterized based on the temperature and type of media they grow in. After the separation and isolation process of the microorganisms, colonial morphology and Gram staining was performed (Appendix II).

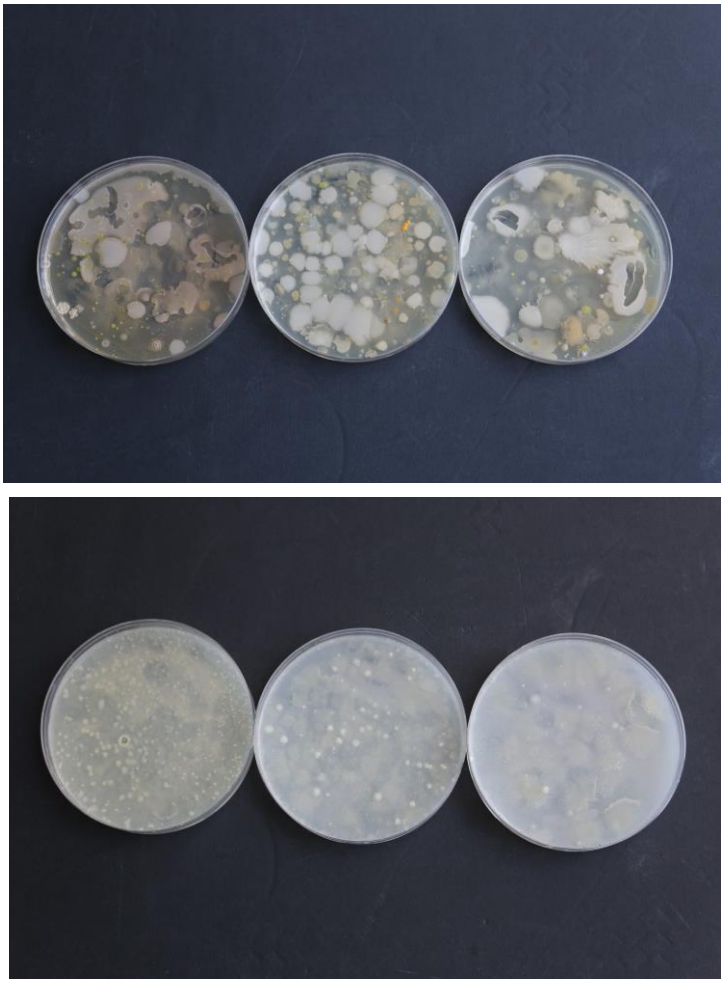

Figure 4: Formation of different bacterial colonies after serial dilution and incubation for 5 days at 28°C

3.1.2 Polyamines production test

Each bacterial strain was streaked on a separate plate of Moeller's decarboxylase agar medium (MDAM) to test the polyamine production ability of the bacterial strain. After 12 hours incubation at 28 ± 2°C, the color intensity was noted to increase gradually with time and the strains that had the bigger stain zones were selected for inoculation (Figure 5).

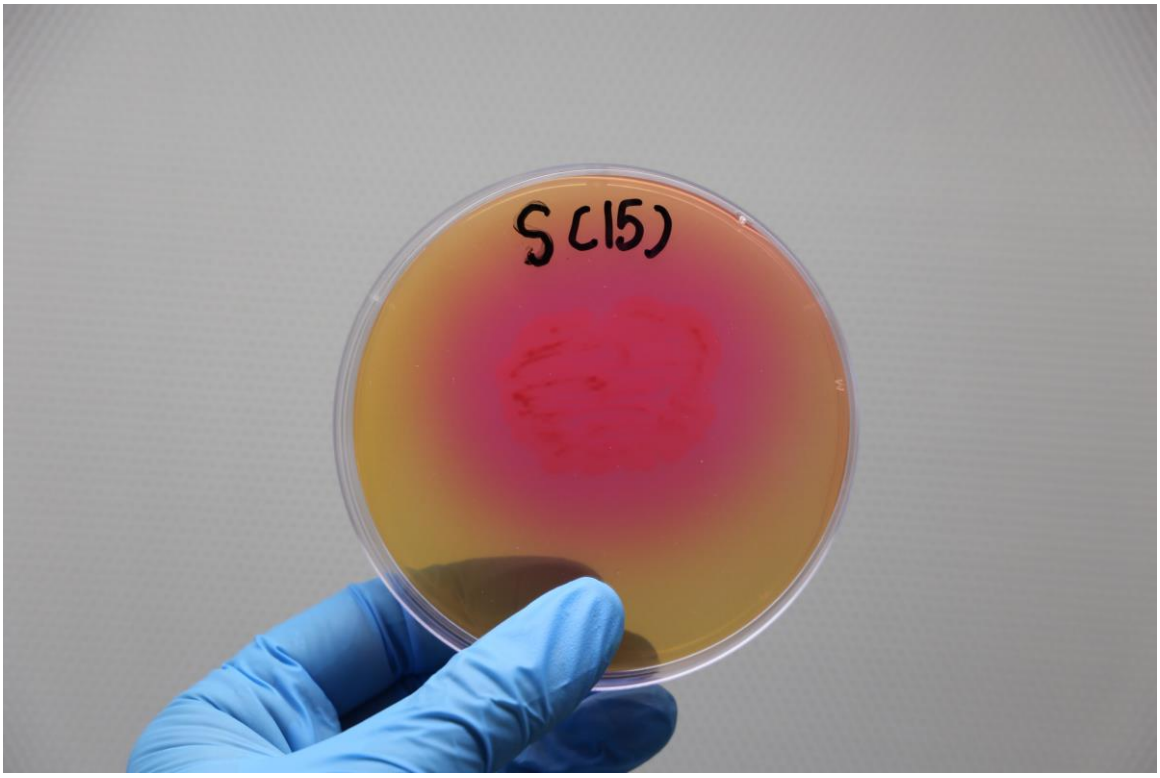

Figure 5: Polyamine production test after streaking on MDAM media and incubation at 28 ± 2°C for 12 hours. Rhizosphere strain S.15.

3.1.3 Phosphorus solublization test

Each bacterial strain was streaked on a separate plate of Pikovskaya agar medium (PVK) to test the phosphorus solubilizing ability of the bacterial strain. After 24 hours incubation at 28 ± 2^{o}C, the clear zones were noted and the strains that had the bigger clear zones were selected for inoculation (Figure 6).

Figure 6: Phosphorus solublization test results of the endophyte strains from Salsola after 24 hours incubation at 28 ± 2^{o}C on (PVK) media.

3.1.4 Auxin production test

After the addition of salkowski reagent to each strain of bacteria, the tubes were incubated at room temperature for 30 minutes and any color change was observed and compared with the control (Figure 7).

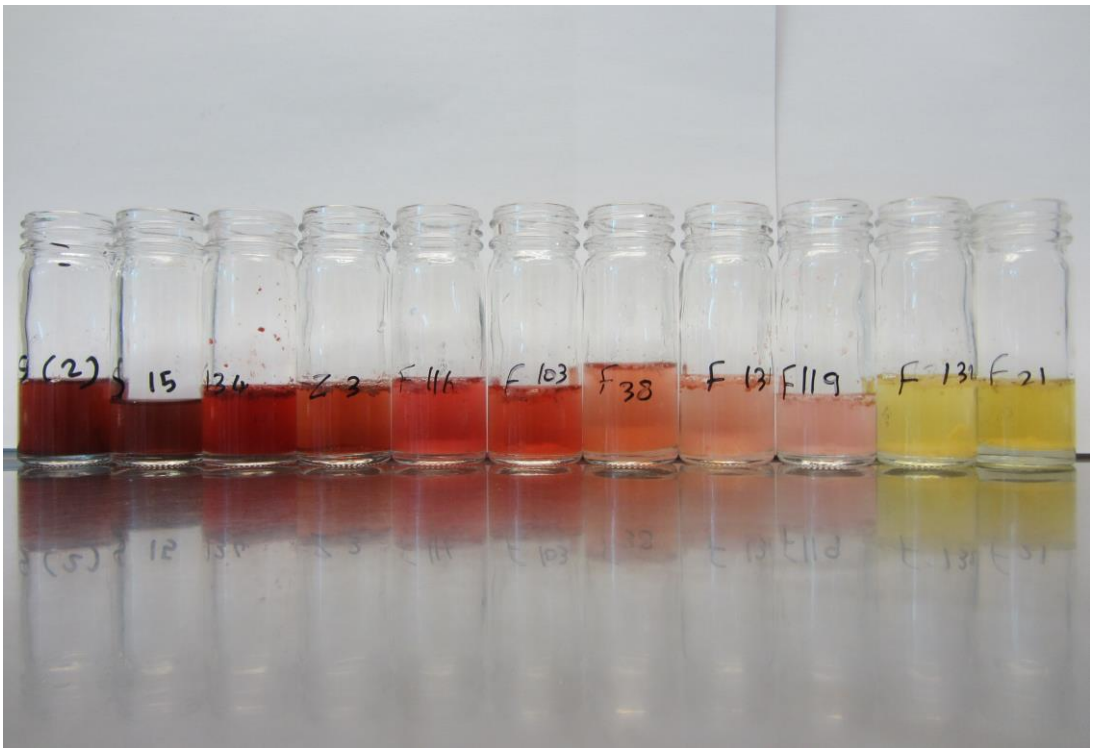

Figure 7: Auxin producing endophytic and rhizosphere strains from weakest to strongest based on the red color intensity.

3.2 Plant growth parameters: *Zygophyllum mandavillei*

3.2.1 Photosynthetic rate

ANOVA analysis revealed a significant interaction in inoculation by water stress ($P<0.01$). Control plants (no inoculation or water stress) had higher photosynthetic rate than plants under water stress with averages of 76% and 71%; respectively. It is believed that the treatment of inoculation was effective on the levels of photosynthetic rates of *Zygophyllum mandavillei* plants under water stress in the first month. During the rest of the experimental period time, there was no significant difference between inoculated and water stressed plants ($P>0.1$) (Figure 8).

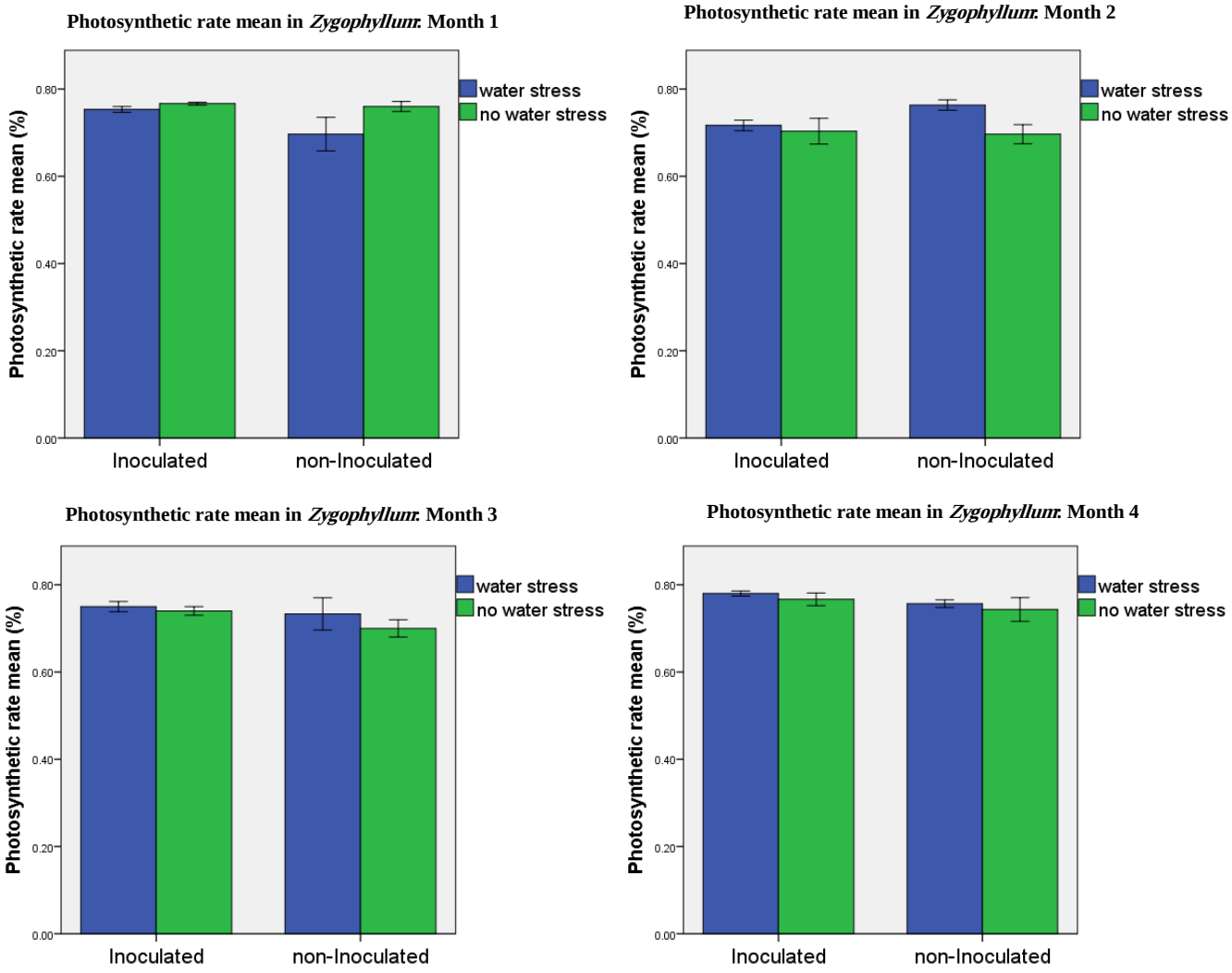

Figure 8: Photosynthetic rate mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.2 Chlorophyll content

In the first month of treatment, the chlorophyll content of control *Zygophyllum mandavillei* plants was less than the inoculated ones, 3.01 and 8.73 respectively. This change is considered significant as (P<0.1). Similar results with significant change in inoculation were recorded during the rest of treatment period. During the third and fourth months of treatment, water stressed plants performed better than the rest of the groups as they had higher chlorophyll content mean (P<0.1) (Figure 9).

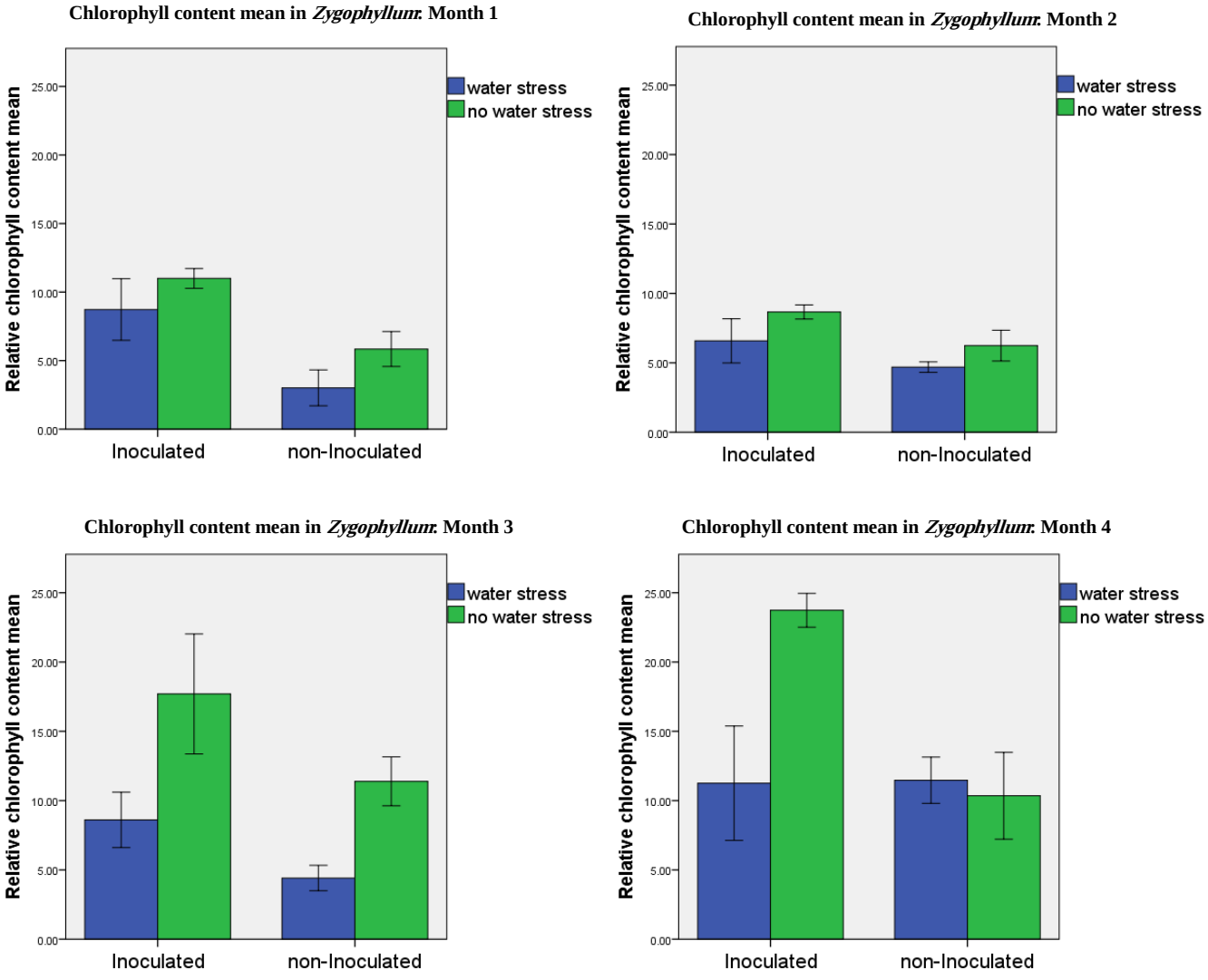

Figure 9: Chlorophyll content mean in 4 groups of Zygophyllum mandavillei with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.3 Root length

Inoculated plants had smaller root length mean than control plants and inoculated water stressed plant had longer roots than control plants in the first and third months (Figure 10). However, during the second and forth months of treatment inoculated plants had longer roots than control plants and inoculated water stressed plants and had longer roots than inoculated plants (P>0.1).

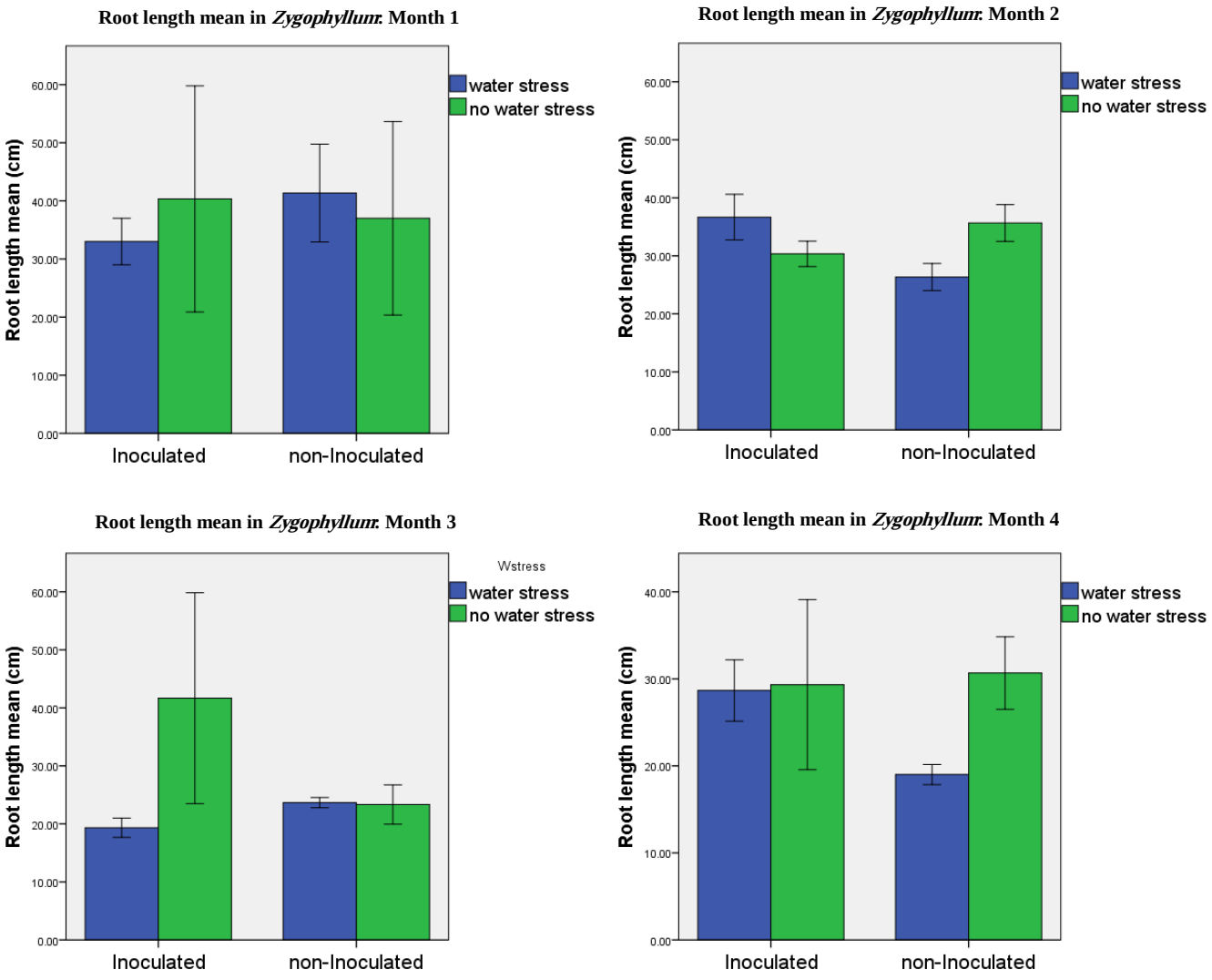

Figure 10: Root length mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.4 Root weight

In the first, third, and forth month of treatment, control plants had bigger root weight than inoculated plants and non-inoculated water stressed plants performed better than inoculated water stressed plants. However, in the second month of treatment, inoculated and water stressed plants performed slightly better than non-inoculated water stressed plants 1.8 g and 1.4 g respectively (Figure 11). The only significant change was during month 3 in water stressed plants ($P<0.1$).

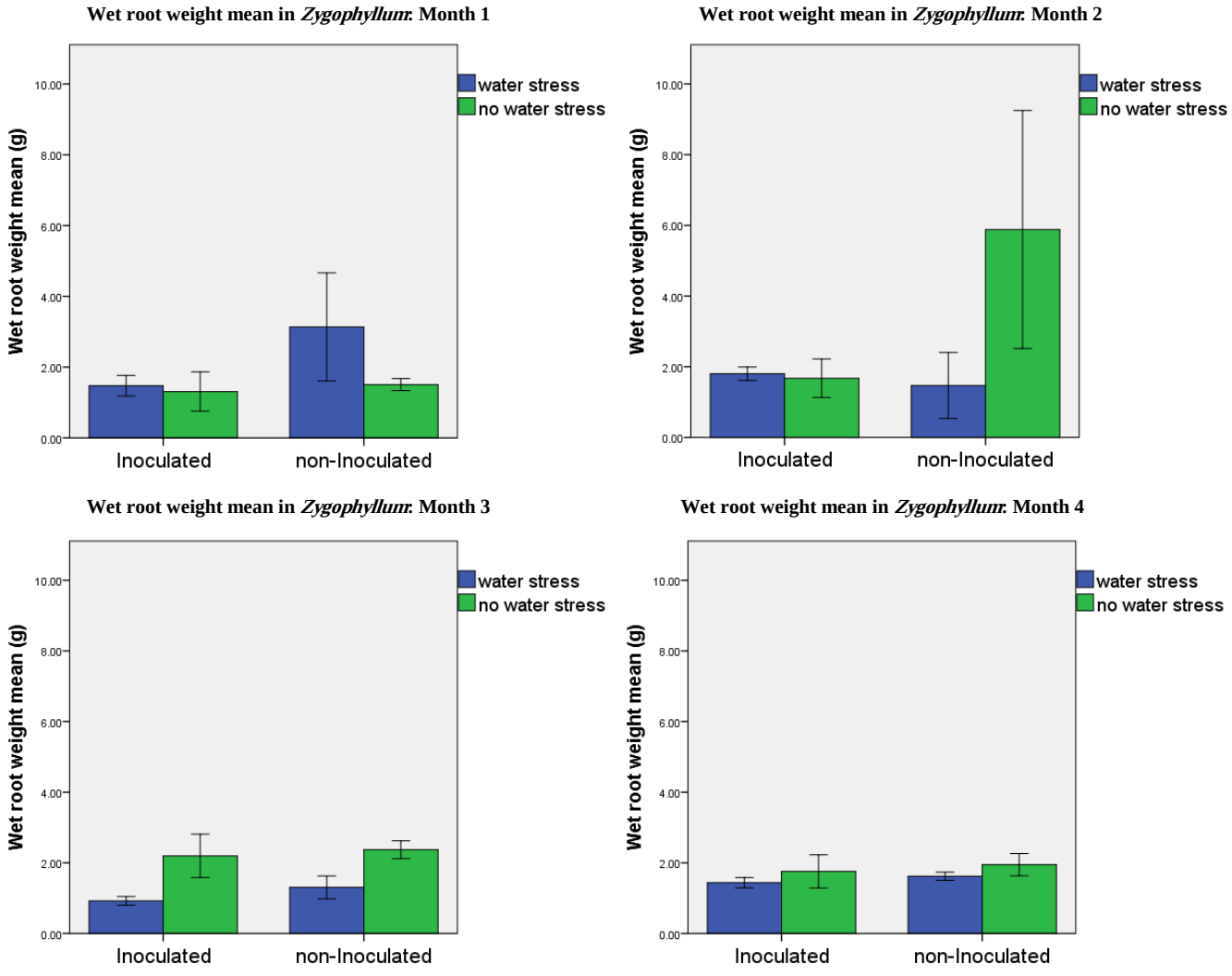

Figure 11: Wet root weight mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.5 Shoot weight

The wet shoot weight of inoculated *Zygophyllum mandavillei* plants was slightly bigger than the control ones, 17.3 g, and 16.4 g respectively. The water stressed plants performed better than the inoculated water stressed plants during the first month of treatment, but this change is not significant as P>0.1. However, during third and fourth months of treatment inoculated and water stressed plants performed better than the non-inoculated one and this change was significant (P<0.1) (Figure 12).

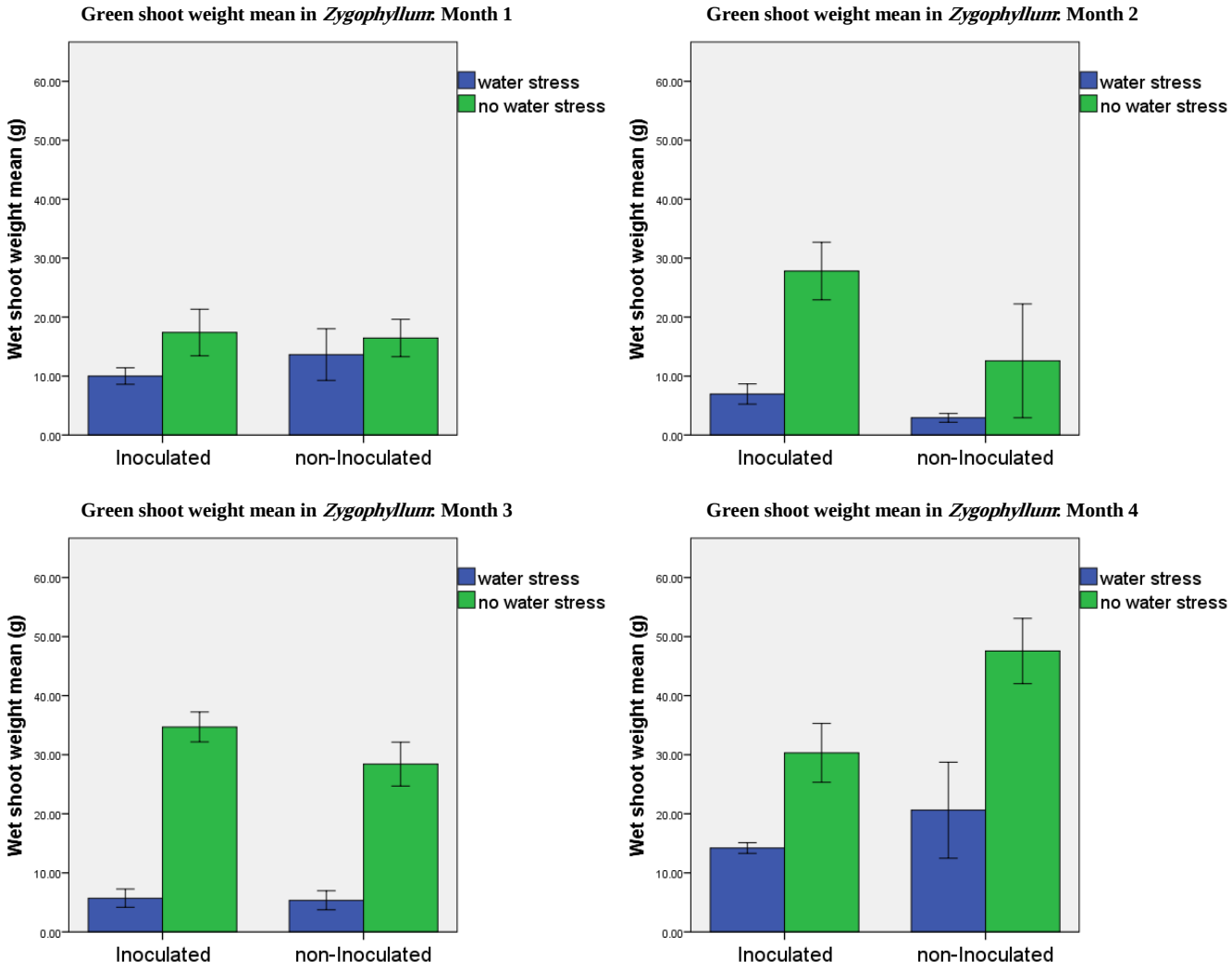

Figure 12: Green shoot weight mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.6 Dry root weight

Control plants had a larger mean of dry root weight than inoculated ones, and non-inoculated water stressed plants had higher mean than inoculated water stressed plants in the first month of treatment, 1.08 g and 0.88 g respectively. Inoculated water stressed plants had a larger mean than non-inoculated water stressed plants, 0.8 g and 0.5 g respectively. Similar results were recorded during the second and fourth months. However, during the third month pf treatment there was a significant difference in water stressed plants (P<0.1) (Figure 13).

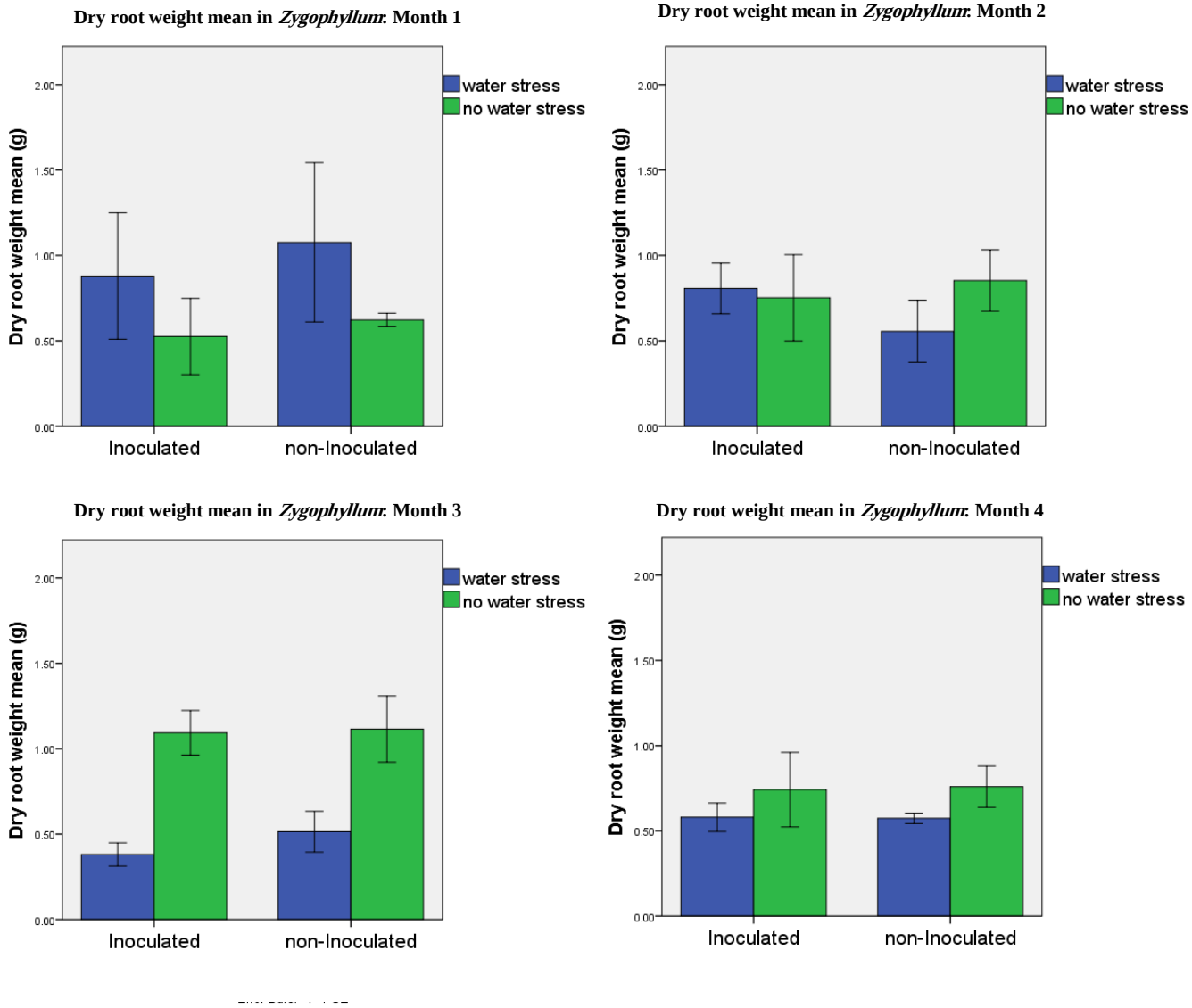

Figure 13: Dry root weight mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.7 Dry shoot weight

During the first month of treatment, control *Zygophyllum mandavillei* plants had slightly larger dry shoot rate than control ones, 2.72 g and 2.3 g respectively. This change was not significant as P>0.1. Similar results were observed in the fourth month of treatment. The inoculation beneficial in the second month as the mean of inoculated water stressed plants was larger, 1.6 g and 0.9 g respectively. During month 3 the mean of water stressed plants was larger than the rest of the groups. The changes in month 3 and 4 were considered significant as P< 0.1 (Figure 14).

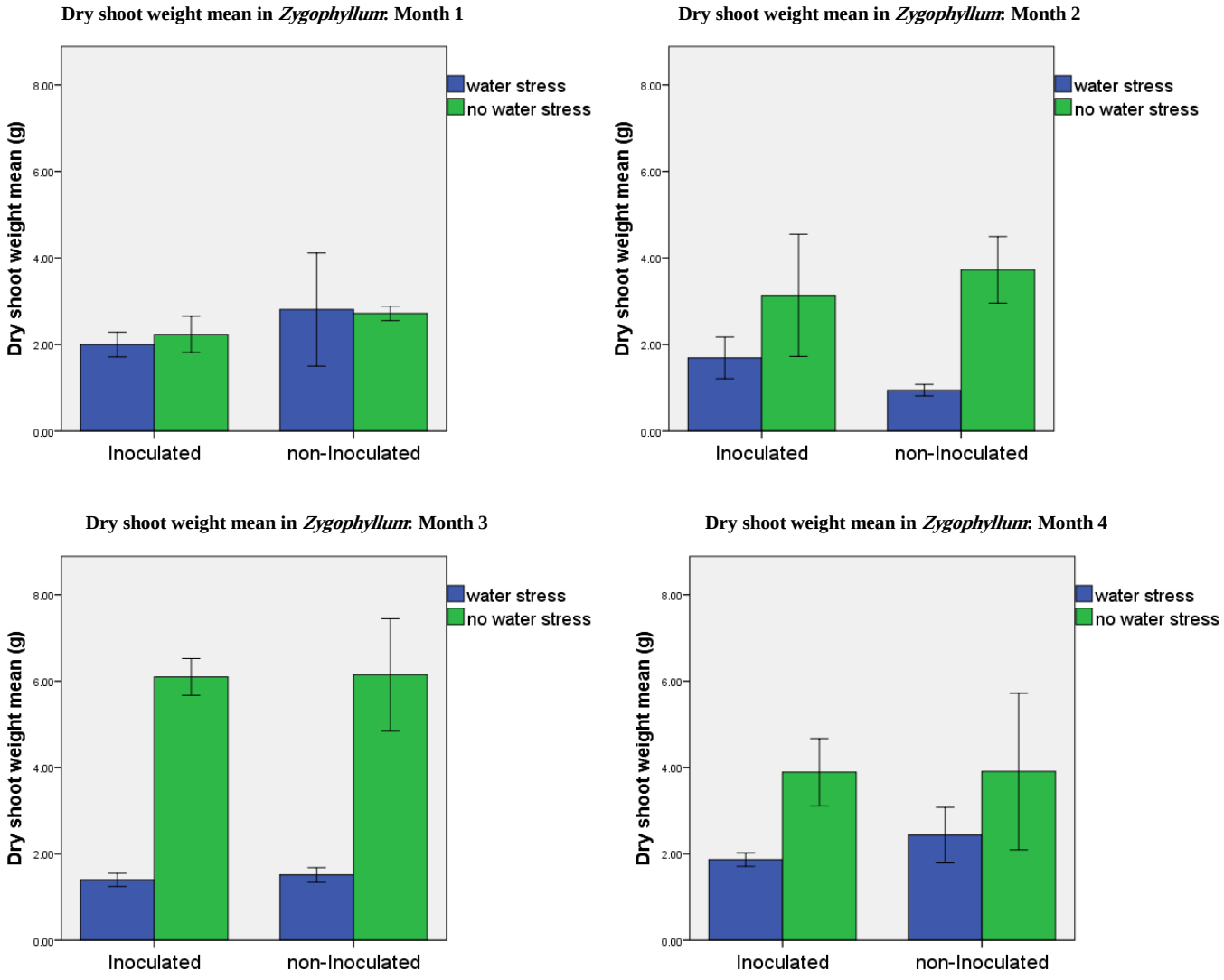

Figure 14: Dry shoot weight mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.2.8 Percent shoot growth

Inoculation didn't have a large impact on the growth percentage of *Zygophyllum mandavillei*. The growth percentage of inoculated plants was larger in the second month of treatment only while the control was higher during the rest of the months. Inoculated water stressed plants performed better than water stressed plants during the third month, 26.1% and 25.7% respectively (P>0.1) (Figure 15).

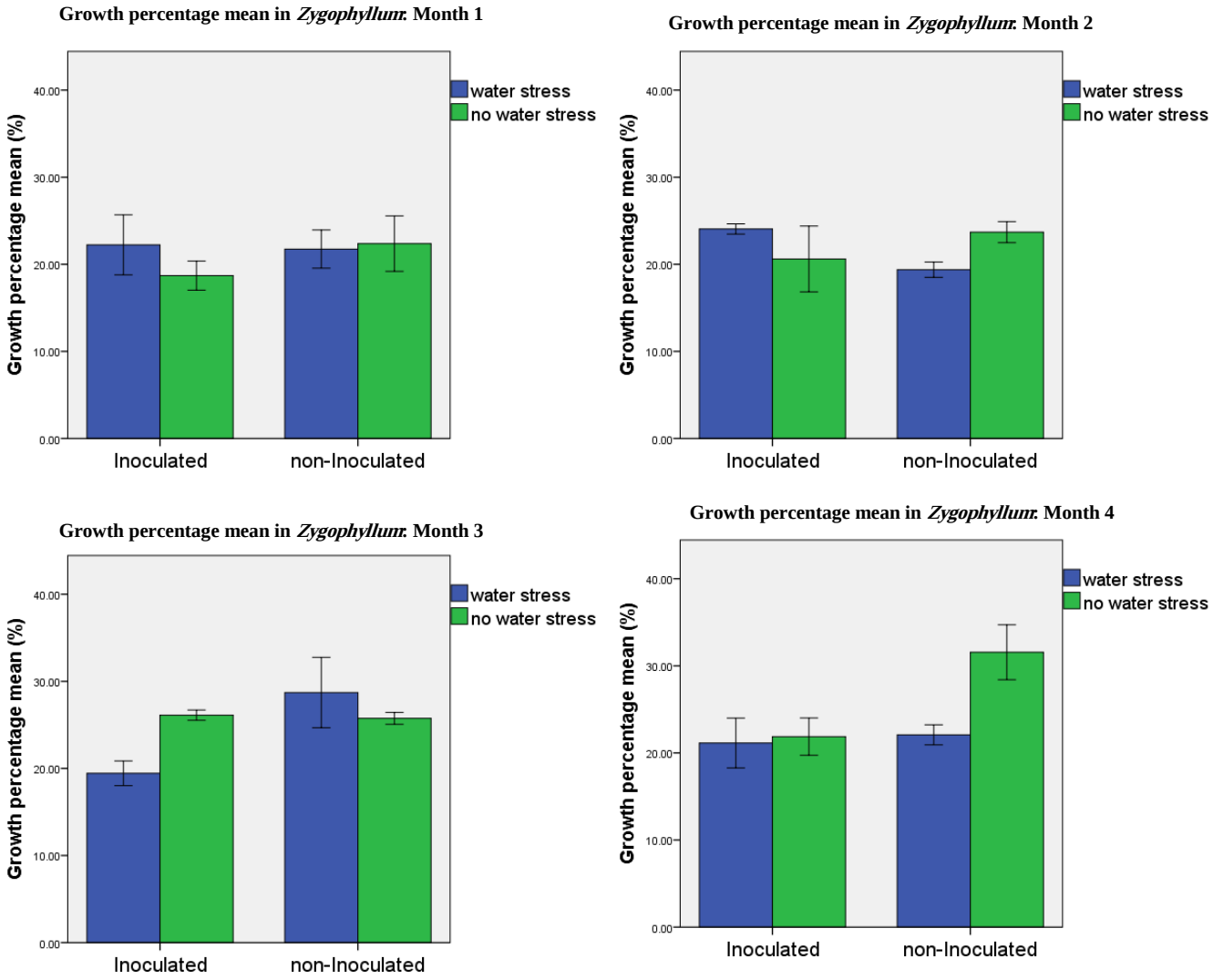

Figure 15: Growth percentage mean in 4 groups of *Zygophyllum mandavillei* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3 Plant growth parameters: *Salsola imbricata*

3.3.1 Photosynthetic rate

No significant difference in photosynthetic rate was found during the first month of treatment (P>0.1). Water stress had a significant difference among groups in the rest of the months (P<0.1) and the means of water stressed plants were higher than control ones (Figure 16).

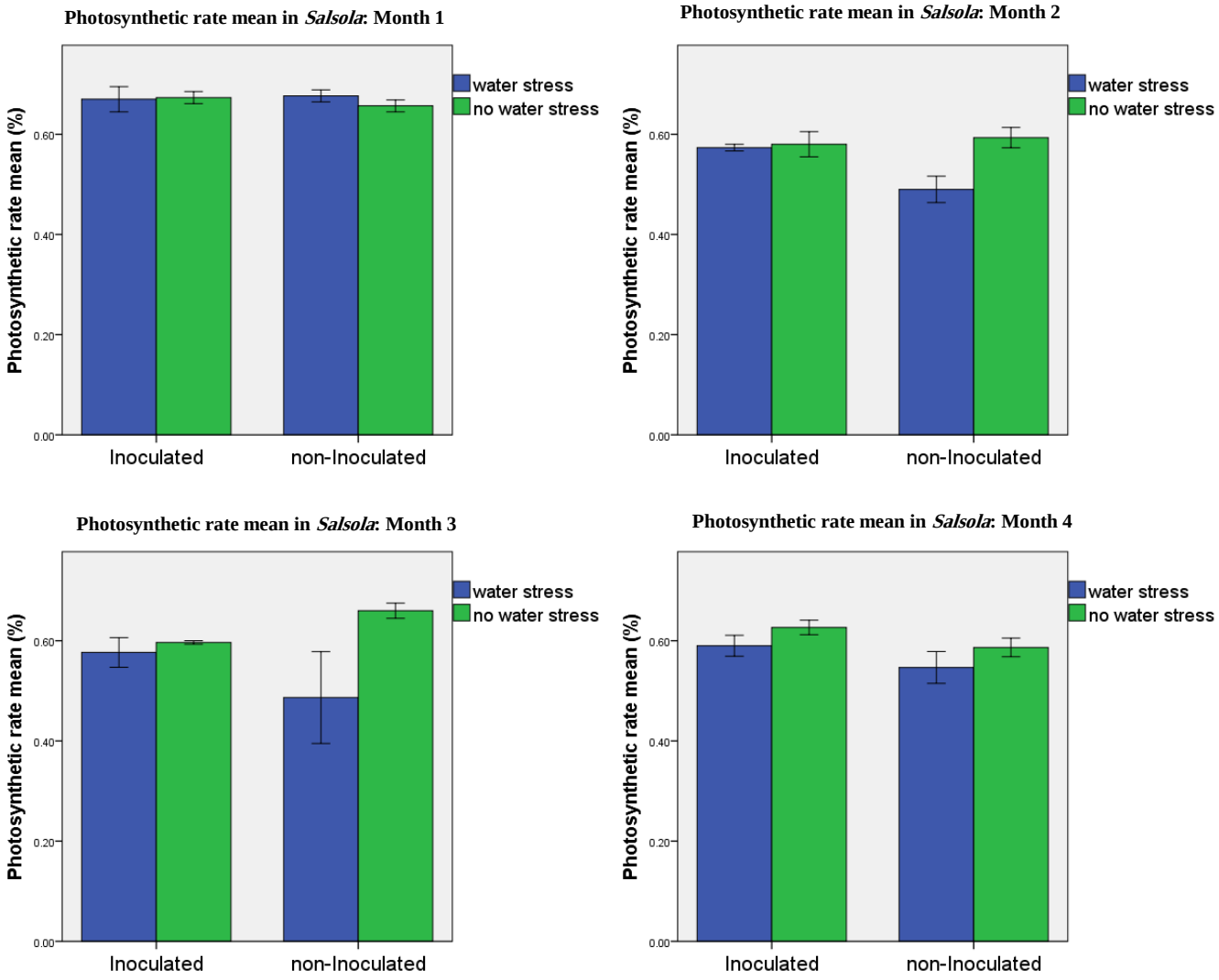

Figure 16: Photosynthetic rate mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.2 Chlorophyll content

During the first month, there was a significant difference in both the inoculation and water stress groups. Non-inoculated and water-stressed *Salsola imbricata* performed better than inoculated and water-stressed ones. Non-inoculated plants had larger chlorophyll content than inoculated plants. The means between groups in the second and fourth months were not significant, but during the third month of treatment there was a significant difference and in last month of treatment, control and water stressed plants had higher means of chlorophyll content. However, in third month it was observed that inoculated plants performed better and had higher chlorophyll content (Figure 17).

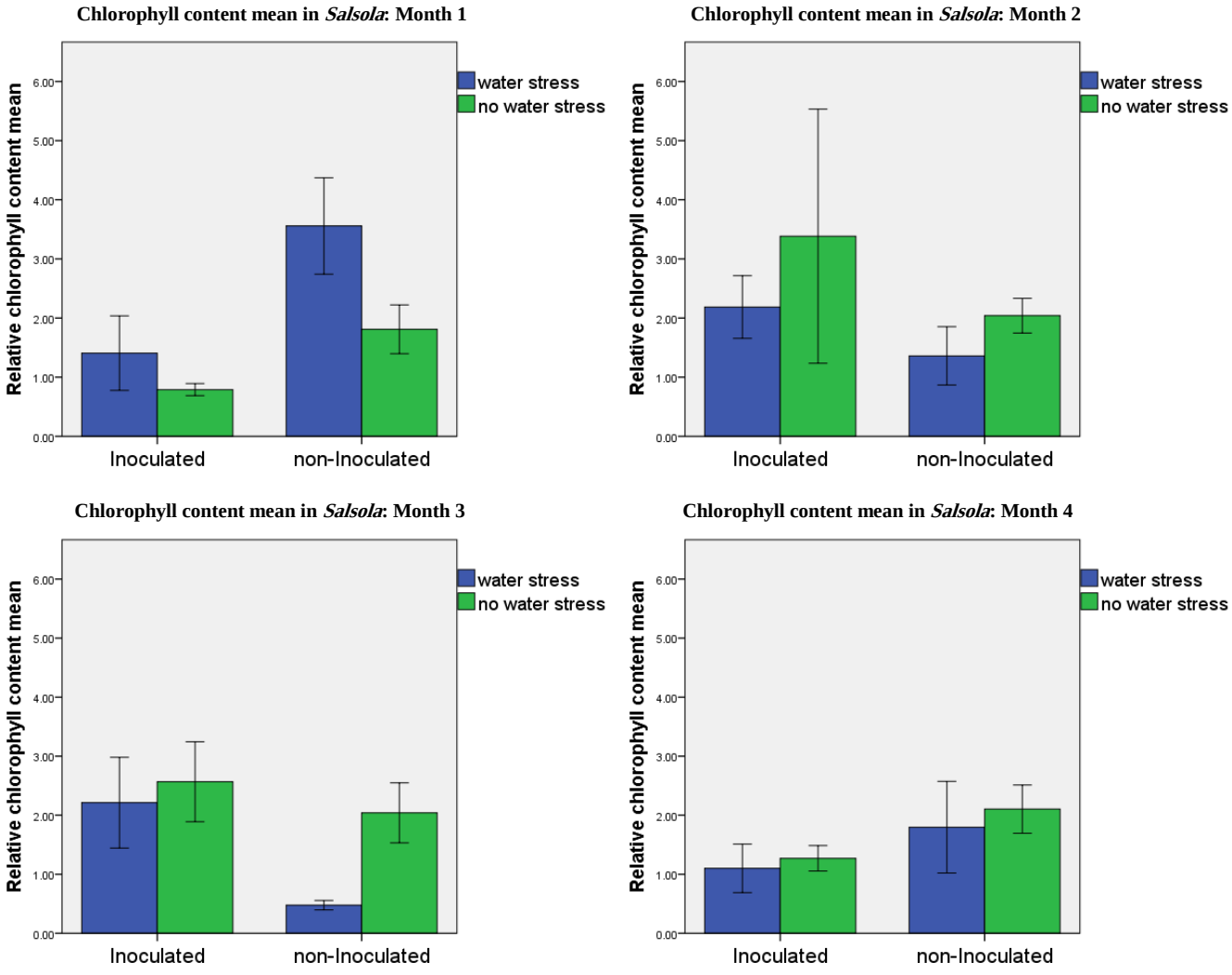

Figure 17: Chlorophyll content mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.3 Root length

Inoculated *Salsola imbricata* plants had bigger root length mean than the control ones during the last three months of treatment. Impact of inoculation on water stressed plants was noted after the first month of treatment, and inoculated water stressed plants had higher means than plants without water stress (P>0.1). The only noted significant difference was in month 3 where non-inoculated water stressed plants had higher means of root length than all groups (35 cm) (Figure 18).

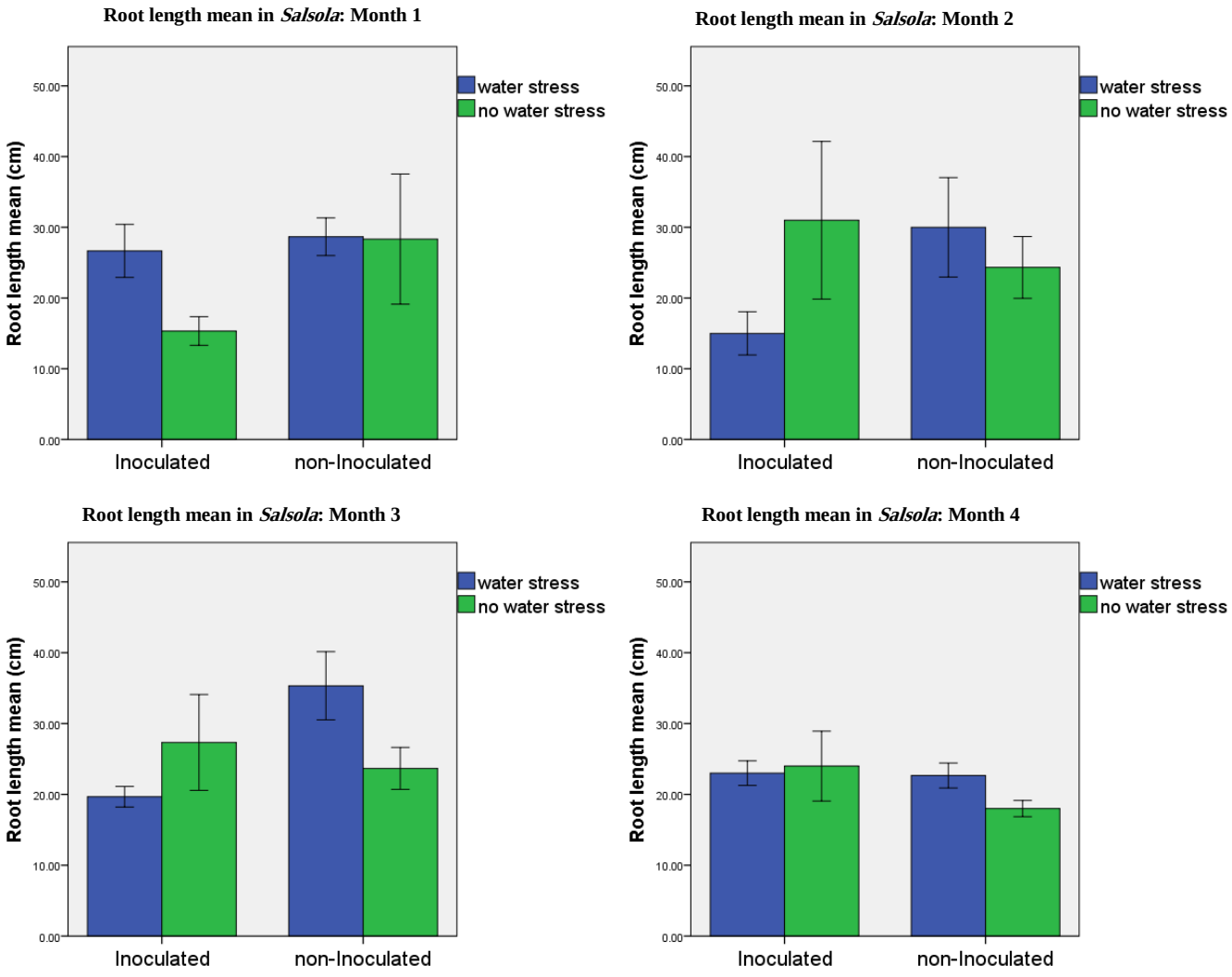

Figure 18: Root length mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.4 Root weight

Impact of inoculation was not observed during the first two months as non-inoculated and water stressed plants had smaller wet root mean than control ones and these changes were not significant. In the third month of treatment, inoculated plants weighed more than control ones (0.47 g, 0.26 g respectively) and inoculated water stressed *Salsola imbricata* weighed more than plants without water stress (0.42 g, 0.37 g). Similar results were recorded in month 4, where inoculated water plants had larger means than the rest of the groups. The changes in month 3 and 4 are considered significant as P<0.1 (Figure 19).

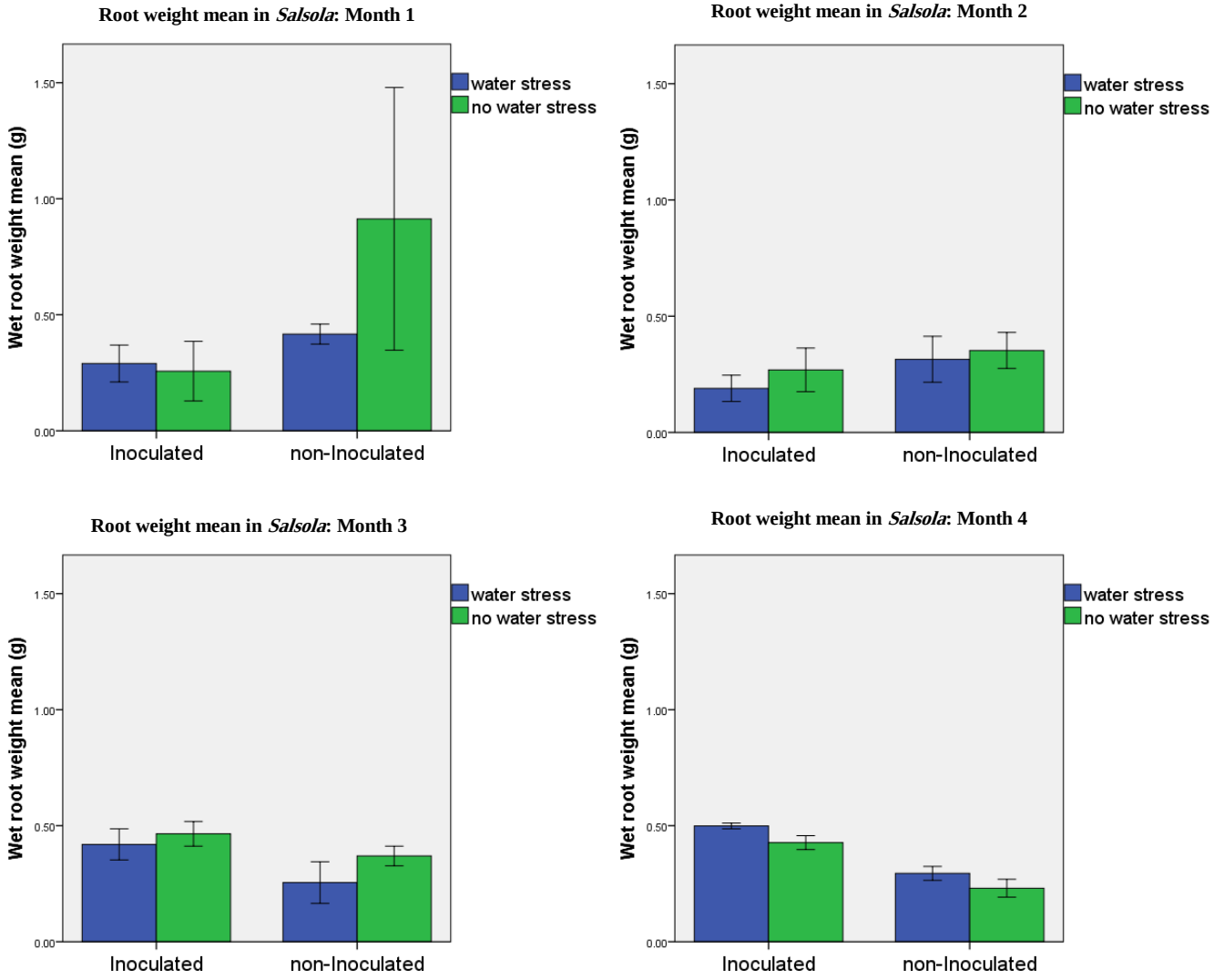

Figure 19: Root weight mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.5 Shoot weight

Inoculated and water stressed plants performed better than control ones during the last three months of treatment. No significant changes were observed ($p>0.1$) in water stress during the first month of treatment. Inoculation was noted to be effective during month 1 and 4 months of treatment as inoculated *Salsola imbricata* had bigger wet shoot weight than control ones (5.18 g, 3.03 g and 0.50 g, 0.29 g respectively). Significant change in water stress was observed during month 3 and water stressed plants had higher wet shoot mean than the rest of the groups (Figure 20).

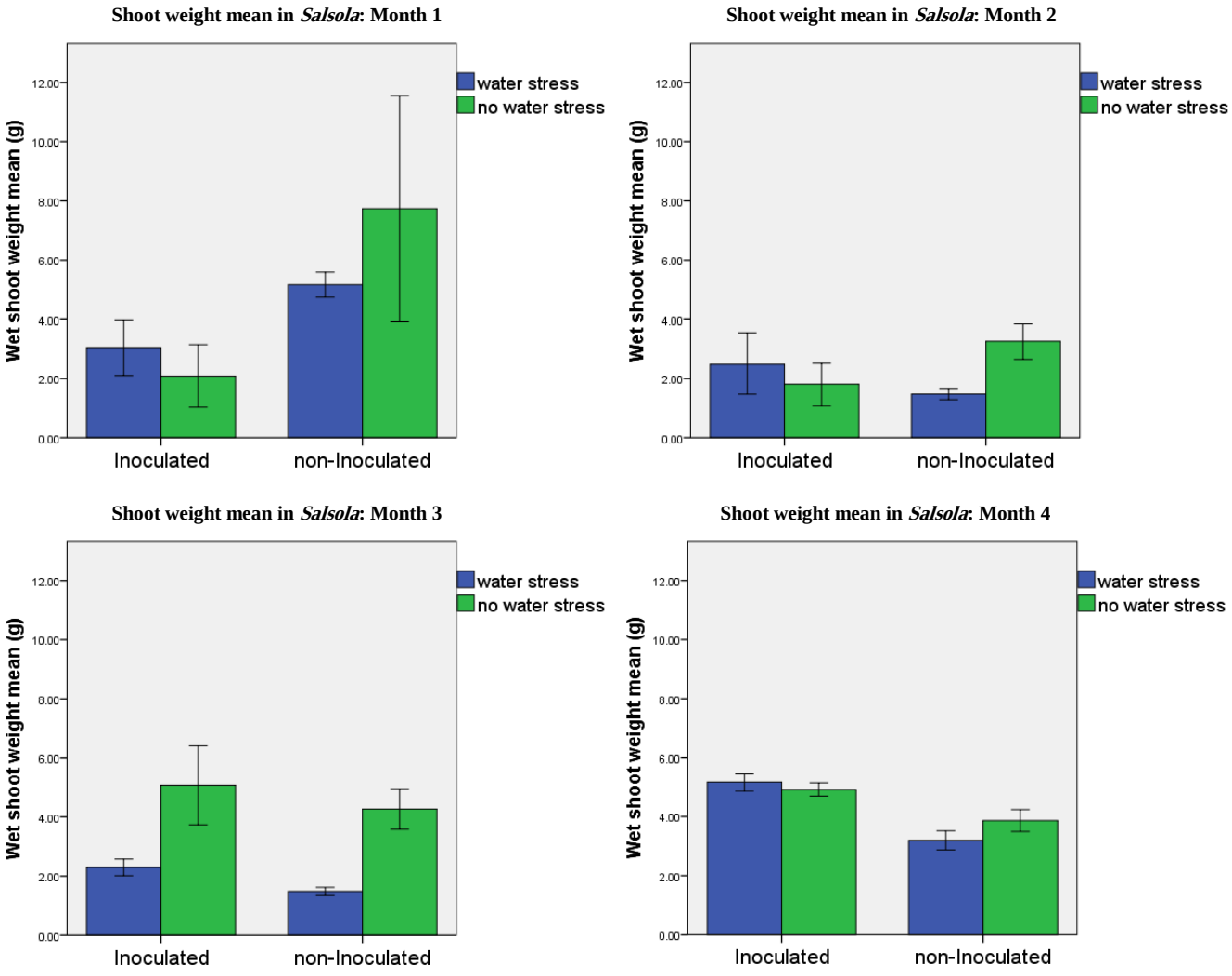

Figure 20: Green shoot weight mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.6 Dry root weight

Inoculation significantly improved the growth of water stressed *Salsola imbricata* during the second and forth months of treatment while it didn't in the first and third months ($P<0.1$). In month 1 and 4, inoculation did not have an impact on the dry root weight mean on Salsola as non-inoculated plants had larger dry root weight mean than control ones (Figure 21).

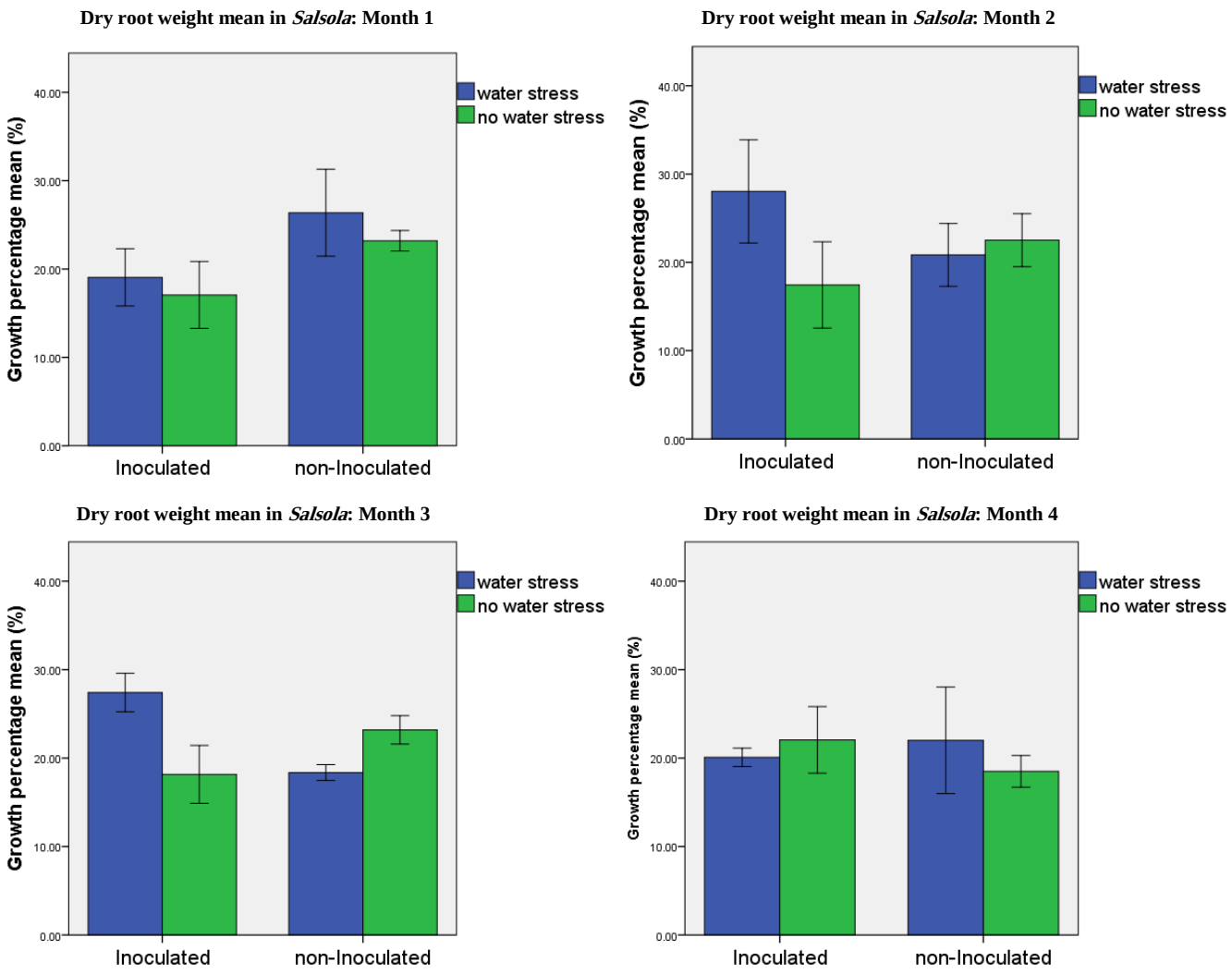

Figure 21: Dry root weight mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.7 Dry shoot weight

The dry shoot weight of *Salsola imbricata* was gradually increasing with every month of treatment in inoculated plants, and during the fourth month it had larger dry shoot weight than the control ones, 1.2 g and 0.8 g respectively. The inoculation on water stressed plants was observed during the second and third month of treatment, however these differences in weight are not significant at P<0.1 (Figure 22).

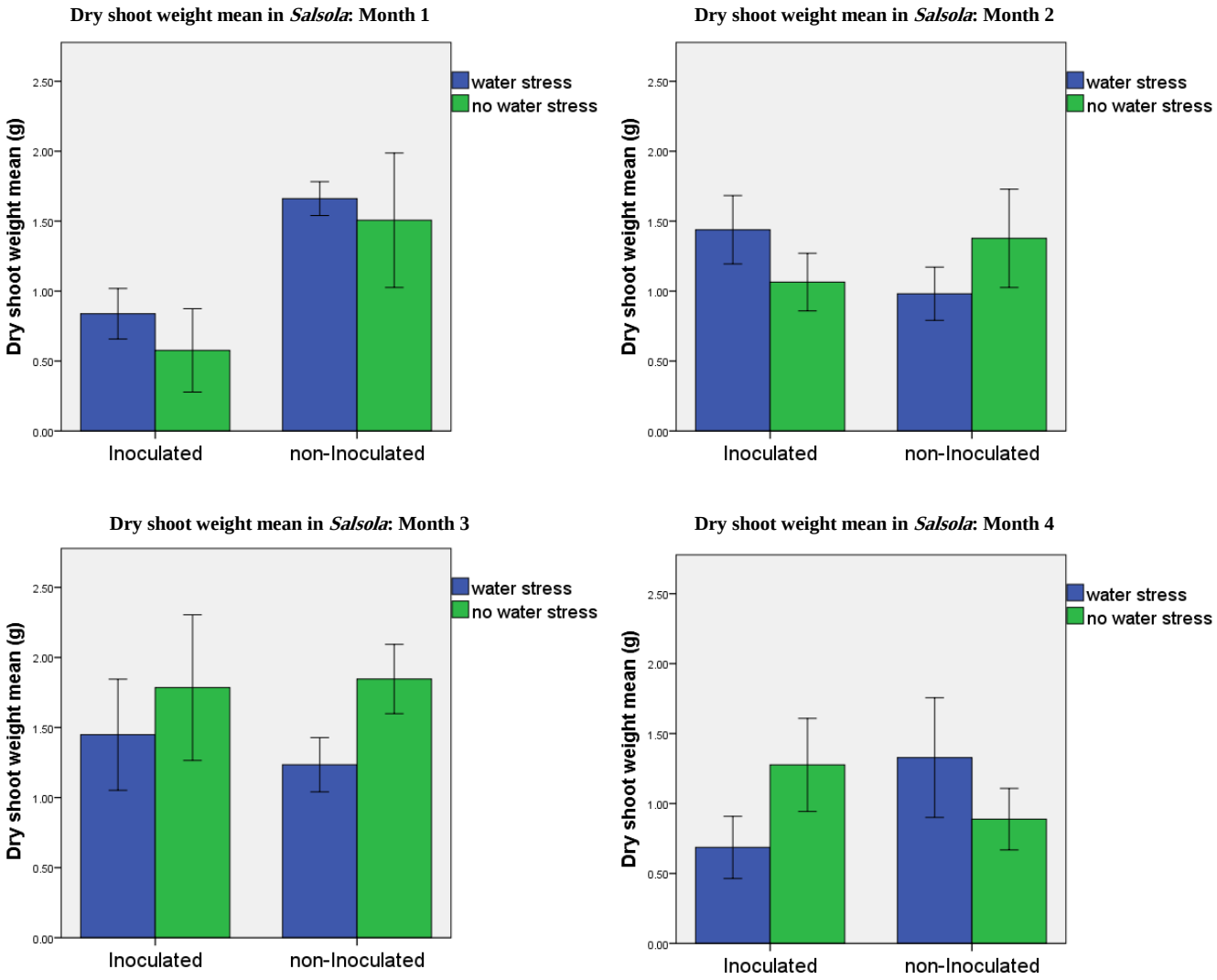

Figure 22: Dry shoot weight mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.3.8 Percent shoot growth

The dry shoot weight of *Salsola imbricata* was gradually increasing with every month of treatment in inoculated plants, and during the fourth month it had larger dry shoot weight than the control ones, 1.2% and 0.8% respectively. However, this change was not significant (P<0.1). A significant impact of inoculation on water stressed plants was observed during the second and third month of treatment and the means were 27.41% and 18.37% in inoculated and non-inoculated plants respectively (Figure 23).

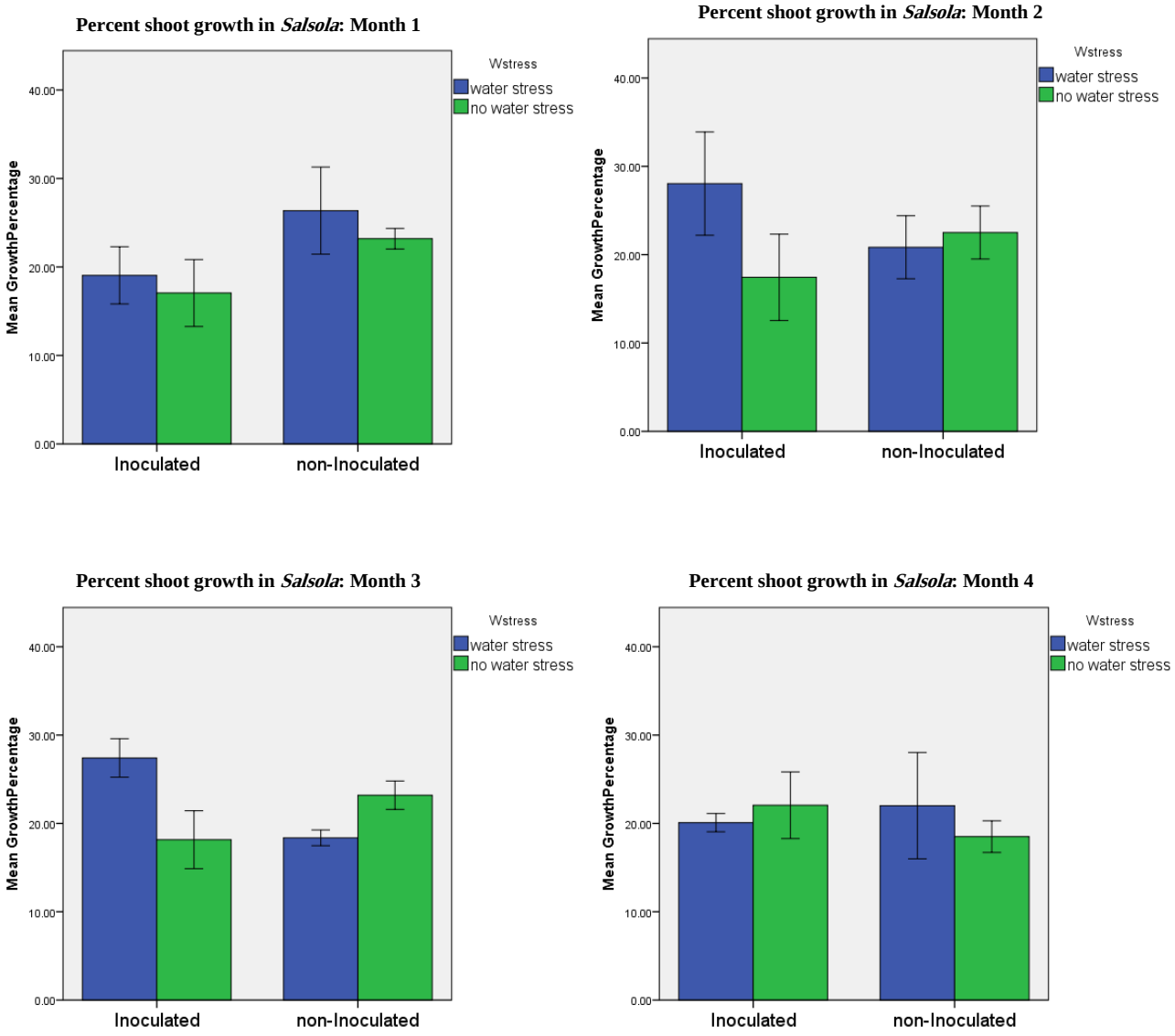

Figure 23: Shoot growth percentage mean in 4 groups of *Salsola imbricata* with different treatments during 4 months of inoculation. The values on the bars are means of 3 replicates.

3.4 Microbial activity

3.4.1 *Zygophyllum mandavillei*

After 4 months of inoculation, the means of microbial activity in inoculated soils of *Zygophyllum mandavillei* were bigger than the non-inoculated soils, 98.29 µg and 45.58 µg, 91.86 µg and 36.77 µg respectively in water-stressed plants and control plants. The differences are significant at P<0.1 (Figure 24).

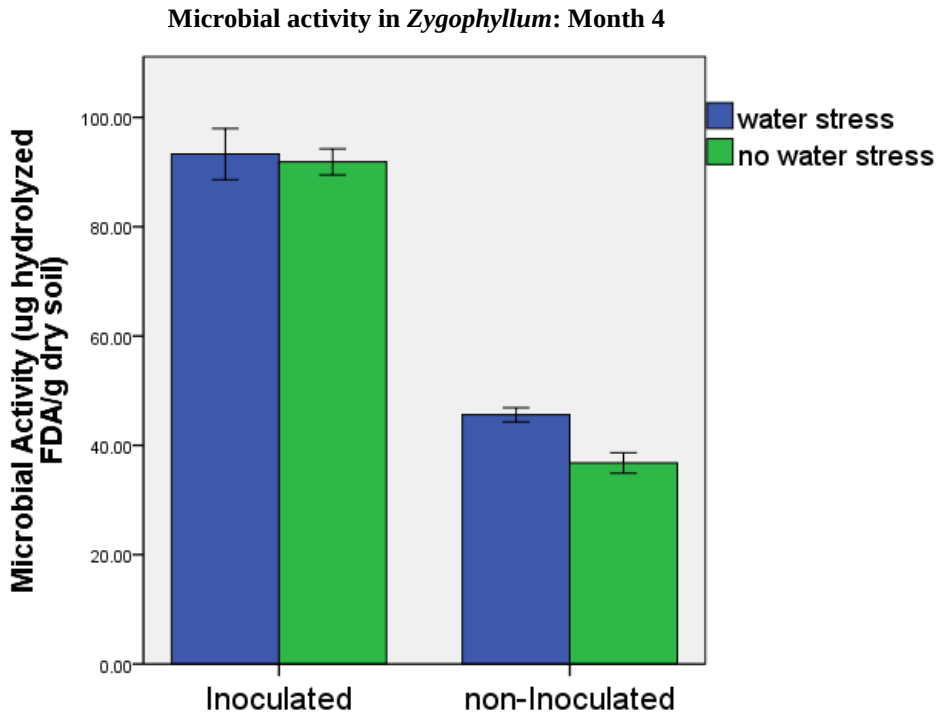

Figure 24: Microbial activity mean in *Zygophyllum mandavillei* soils after four months of inoculation. The values on the bars are means of 3 replicates.

3.4.2 *Salsola imbricata*

There was a significant difference in the means of microbial activity in *Salsola imbricata* in both inoculation and water stress after four months of treatment ($P<0.1$). Microbial activity in inoculated soils was larger than the control, 76.01 µg and 26.28 µg respectively. Similarly, inoculated water stressed plants had larger means than water stressed non-inoculated plants, 85.03 µg and 45.58 µg respectively (Figure 25).

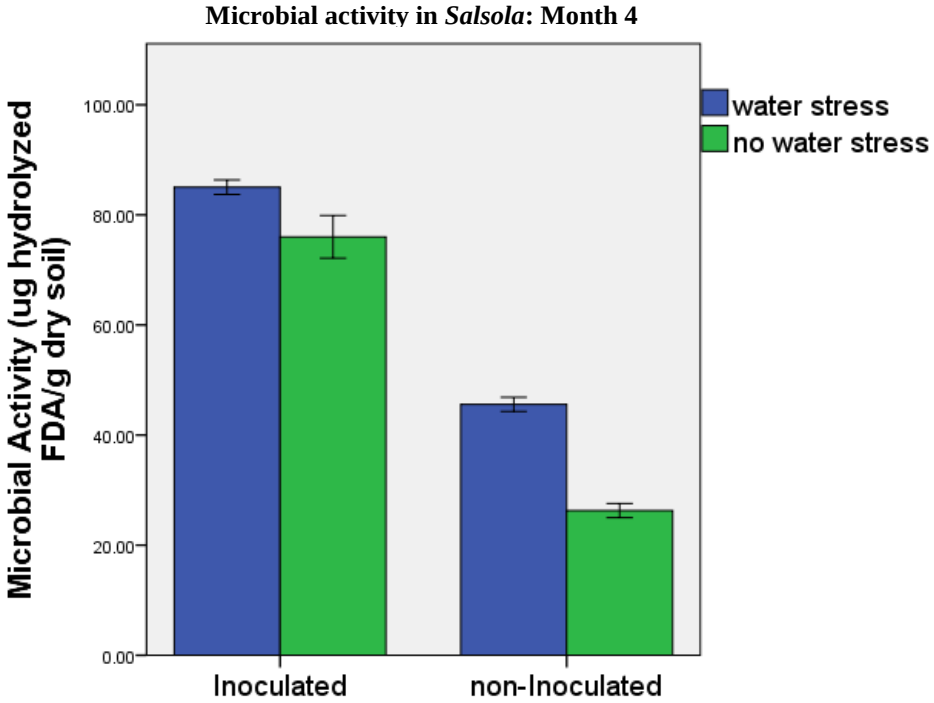

Figure 25: Microbial activity mean in *Salsola imbricata* soils after four months of inoculation. The values on the bars are means of 3 replicates.

3.5 Carbon sequestration

3.5.1 *Zygophyllum mandavillei*

There is no significant effects of inoculation on organic carbon content for *Zygophyllum mandavillei* (P>0.1). The average carbon sequestration potentials of non-inoculated and water stressed plants were higher than the inoculated ones under no water stress, 72.78% and 66.15% respectively (Figure 26).

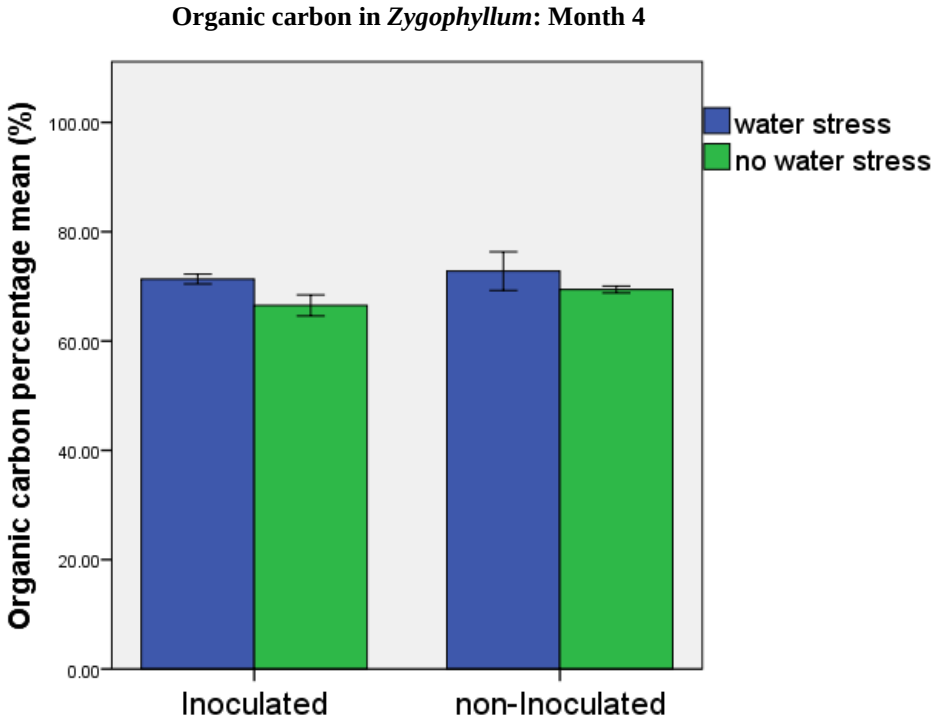

Figure 26: Statistical analysis of total organic carbon content in *Zygophyllum mandavillei* plants after 4 months of inoculation. The values on the bars are means of 3 replicates.

3.5.2 *Salsola imbricata*

The non-inoculated treatments in *Salsola imbricata* had a higher carbon content mean than the inoculated plants, 79.5% and 72.6% respectively. This indicated that the inoculation did not improve the carbon sequestration in *Salsola imbricata* (Figure 27).

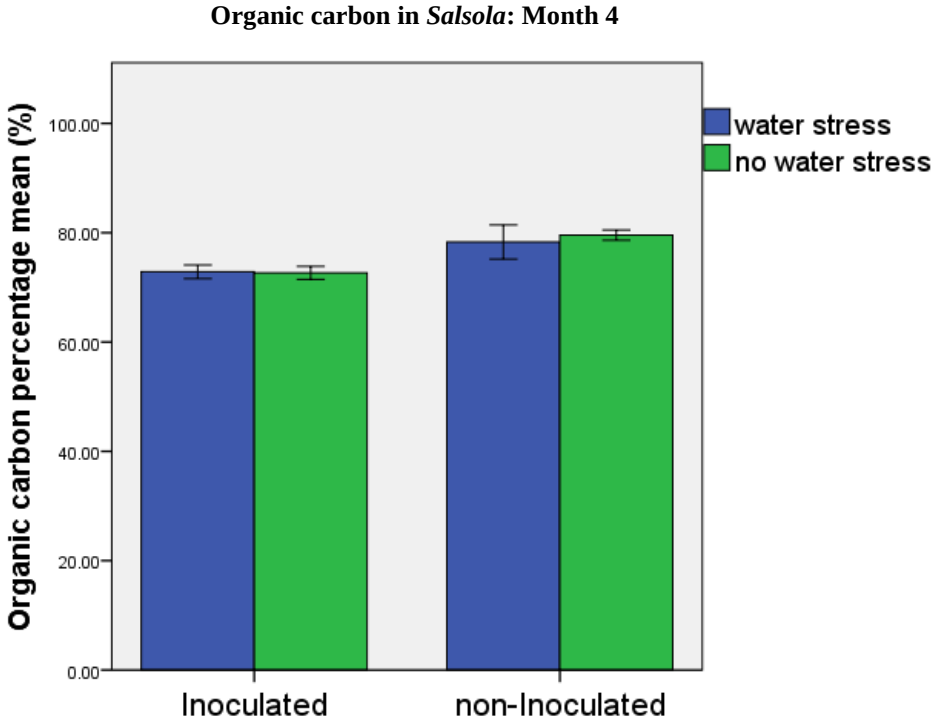

Figure 27: Statistical analysis of total organic carbon content in *Salsola imbricata* plants after 4 months of inoculation. The values on the bars are means of 3 replicates.

Chapter 4: Discussion

No previous work on impacts of microbial inoculation was reported on the two UAE native species *Salsola imbricata* and *Zygophyllum mandavillei*. It was the aim of the current study to isolate plant growth-promoting bacteria (PGPB) from those two species without the use of the well-known ones and to analyze the eco-physiological growth of plants under the effect of these native PGPB. The plants' carbon sequestration was also closely monitored. Ultimately, these enhanced plants will grow faster and have a positive impact on desert ecosystems and improve their quality.

In the present study, direct growth attributes like shoot weight, root weight, and root length were improved by the inoculation on *Salsola imbricata.* Inoculation was effective in *Salsola imbricata* plants in both water stressed and plants without water stress. The fresh root weights in inoculated *Salsola imbricata* were heavier than the rest of the groups after 3 months of inoculation. The inoculation improved the shoot weigh of fresh *Salsola imbricata* after four months of inoculation and similar improvement were also noted in dry root and shoot weight after 2-4 months of inoculation. Root length improved after two months of inoculation, and it was improved in water stressed plants as well.

Beneficial free-living soil and rhizosphere bacteria are often referred to as plant growth-promoting rhizobacteria (PGPR) or plant growth-promoting bacteria (PGPB) and are found in a close association with the root surfaces of many different plants (Lucy, *et al*., 2004). However, to be inclusive of the many different types of bacteria that facilitate plant growth, the term plant growth-promoting bacteria (PGPB), is preferred (Bashan & Holguin,1998). Moreover, while numerous free-

living soil bacteria are considered to be PGPB, not all bacterial strains of a particular genus and species have identical metabolic capabilities. For example, some *Pseudomonas putida* strains may actively promote plant growth while others have no measurable effect on plants (Glick *et al.*, 2007). PGPB can function either indirectly or directly (Glick, 1995; Glick *et al.*, 2007). Indirect mechanisms of promotion of plant growth by PGPB are those related to the production of metabolites, such as siderophores which can sequester iron necessary for the growth of pathogens (Matthijs *et al.*, 2007) and antifungal metabolites (El-Tarabily *et al.*, 2010) which increase plant growth by decreasing the activities of pathogenic fungi and bacteria by any one or more of several different mechanisms such as production of antibiotics and cell-wall degrading enzymes (Glick *et al.*, 2007). Direct plant growth promotion by PGPB generally provide the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment (Glick *et al.*, 2007).

Direct promotion of plant growth can occur in several different ways. PGPB may (1) fix atmospheric nitrogen and supply it to plants (Dobbelaere *et al.*, 2003); (2) synthesize and secrete siderophores which can solubilize and sequester iron from the soil and provide it to plant cells (Matthijs *et al.*, 2007); (3) synthesize different phytohormones or plant hormones or plant growth regulates (PGRs) including auxins, cytokinins, gibberellins and polyamines which can directly enhance various stages of plant growth (Kuklinsky-Sobral *et al.*, 2004; Nassar, *et al.*, 2003); (4) solubilize minerals such as phosphorus which then become more readily available for plant growth (Rodríguez & Fraga, 1999); (5) by stimulation of ion uptake or transport systems in plants (Mantelin & Touraine, 2004) and (6) by the synthesis of the enzyme that can modulate plant ethylene levels (Glick, 1995; Glick *et al.*, 2007).

A particular bacterium may affect plant growth and development using anyone, or more, of these mechanisms. Moreover, since many PGPB possess several characters that enable them to facilitate plant growth, a bacterium may utilize different characters at various times during the life cycle of the plant, and may vary considerably in its effectiveness depending upon the plant host and the soil composition (Glick *et al.*, 2007) .

Interestingly, PGPB generally have little or no measurable effect on plant growth when the plants are cultivated in nutrient-rich soil and grown under optimal conditions in the absence of stress (Glick *et al.*, 2007).

Some physiological parameters and growth attributes in *Zygophyllum mandavillei* were also improved by inoculation. Chlorophyll content and photosynthetic rates were significantly different during the four months of treatment. Shoot weight of inoculated plants was larger than the rest of control and water stressed groups after 3-4 months of inoculation. Similarly to *Salsola imbricata*, significant changes in dry root and shoot weights were also observed after 2-3 months. Root length was significantly improved after 2 months of inoculation.

These findings are similar to what was reported by Bai *et al.* (2002) as endophytes were used to improve the growth of plants. Isolated endophytic *Bacillus subtilis* and *B. thuringensis* from surface sterilized soybean root nodules were found to increase soybean weight when plants were inoculated with these endophytic bacteria. Inoculation with the endophytic bacterium *Bacillus mojavensis* increased growth of corn and beans. There was a 70% average increase in root and shoot growth in endophyte inoculated plants compared to the non-inoculated control plants (Bacon & Hinton, 2002).

In *Salsola imbricata*, root length and wet root weight has significant interactions but not with the main effects. Inoculation resulted in larger shoot weights of *Salsola imbricata* after one month of treatment and it also improved the plant growth under water stress after three months of treatment. Moreover, there was a significant difference in inoculation on dry root and shoot weights.

According to previous published literature, it was observed that PGPB had the ability to increase the production of many crops such as sunflowers, soybeans, peanuts, and tomatoes (Nejad & A Johnson, 2000; Bai *et al.*, 2002; Fernando Rojas *et al*., 2012; Prasad & Babu, 2017). PGPB produce important plant hormones and compounds such as auxins and polyamines. They may also have the ability to solubilize phosphorus from the soil, making it more readily available for the plant. Increased amounts of research were made in that field to better understand how they function and interact with plants. Few bacterial strains (such as *Azotobacter*) are well established and are used by many farmers nowadays (Gyaneshwar *et al.*, 2001).

The results of the current study showed that the inoculation had a positive impact on the physiological parameter of both *Salsola imbricata* and *Zygophyllum mandavillei* in the first and second month of inoculation (P= 0.097, P= 0.084). Chlorophyll content in both species was significantly higher in inoculated plants compared to control ones during 1-4 months of treatment. Photosynthetic rate was noted to be higher in both species after 1-3 months of inoculation. Similar results were recorded regarding the impact of inoculation on water stress. Chlorophyll content and photosynthetic rate are important physiological parameters and increased rates of them will improve the plant growth. The endophytic bacterium *Pantoea agglomerans* was reported by Riggs *et al.* (2001) to increase corn productivity, and another four endophytic bacteria isolated from rice roots identified as *Pseudomonas*

fluorescens (S3), *Pseudomonas tolaasii* (S20), *Pseudomonas veronii* (S21), and *Sphingomonas trueperi* (S12) were shown to promote rice growth (Adhikari *et al.,* 2001).

The results obtained in the current investigation varied in morphological parameters of plants, and not all of them were significant. In *Zygophyllum mandavillei*, water stress had a significant impact on wet shoot weight, dry root weight, and dry shoot weight after 2-4 months of inoculation. During the second month of treatment, there was a significant interaction in wet shoot weight between inoculation and water stress but not in the main effects.

Soil microbial activity was performed by the end of the current study to assess whether the inoculation persisted or not. Results from the experiment stated that inoculated soils had more microbial activity than the control ones even after four months of inoculation, meaning that the inoculation was successful and effective. However, significant changes in all physiological and morphological parameters were not observed. The higher microbial activity in the treated soil appears to be related to the greater number of aerobic bacteria and actinomycetes but may also have resulted from the activity of fungi which do not sporulate freely and as a consequence may not have been detected in large numbers on the soil dilution plates. The soil dilution plate technique does not differentiate between fungal colonies arising from hyphae and those from spores as suggested by Swisher and Carroll (1980). Therefore, the numbers of fungi which do not sporulate freely are often underestimated in dilution counts, which may have been the case in the present study.

Swisher and Carroll (1980) developed a method, based on the hydrolysis of fluorescein diacetate (3', 6'-diacetylfluorescein) (FDA) to determine the amount of microbial activity in needle litter, soil and litter. Schnurer and Rosswall (1982); Inbar *et al.* (1991); Boehm and Hoitink (1992) reported the use of FDA hydrolysis to

determine total microbial activity in soil, potting mix and straw litter, respectively. FDA has been used routinely as a vital fluorescent stain for soil fungi (Söderström, 1979) FDA, a non-fluorescent substrate, is hydrolysed by various enzymes (such as proteases, lipases and esterases) of living cells and yields fluorescein (Rotman & Papermaster, 1966). Fluorescein remains in the cell causing intracellular fluorescence which can be visualised by fluorescence microscopy and can also be quantified by fluorometery or spectrophotometry. Earlier studies have shown that activity of all fungi investigated (Söderström, 1977), most bacteria (Lundgren, 1981), and some protozoa and algae (Medzon & Brady, 1969) could be assayed with FDA hydrolytic activity. The FDA hydrolysis technique can be considered as simple, inexpensive, and an accurate reflection of the activity of most microbes (Schnürer & Rosswall, 1982).

A possible explanation to the fluctuation in results is the microbial compatibility within the selected strains of bacteria. After microbial compatibility test was demonstrated *in vitro* in nutrient agar plates, it was recorded that some strains (mainly endophytic) were highly competitive against other strains as clear zones were visible on agar plates around bacterial colonies.

Another possibility to explain the difficulty of observing changes in all plant growth parameters is the plants' age. The inoculation was applied on 5 month old plants and this could be a late stage to incorporate the inoculation as the plants had almost fully developed. It is well known that integrated nutrient management systems are needed to maintain agricultural productivity and protect the environment. Microbial inoculants are promising components of such management systems. Studies with microbial inoculants and nutrients have demonstrated that some microbial inoculants can improve plant uptake of nutrients and thereby increase the use efficiency of applied chemical fertilizers and manures and some of

these microbial inoculants on the other hand have no effect at all on plant yield and productivity (Adesemoye & Kloepper, 2009; Khalid *et al*., 2004; Schippers *et al*., 1987).

No previous literature was found regarding halophyte carbon sequestration ability under the impact of inoculation. In the current study it was found that the inoculation did not improve the organic carbon content in both species after inoculation. This could be due to the factor of time as some plants require more time to mature and store carbon within different parts of the plant.

Parmar *et al*. (2016) assessed the effect of long term organic manure application on soil- plant carbon stock and they reported that long term use of farmyard manure showed better yield and greater amount of carbon stock in plants like tomato and cauliflower (Parmar *et al.*, 2016). The aim of using organic manure was to improve the growth and yield of crops. Relating back to the work of this thesis, it could be possible to notice more plant potential to sequester carbon by inoculating the native halophytes for longer periods of time, with the aim of improving their growth.

Chapter 5: Conclusion

In conclusion, halophytes (plants that are able to grow under saline conditions) are vital to desert ecosystems, but they generally grow at a slow rate, especially when under drought conditions. Halophytes have important environmental services and they provide food resources to many desert organisms. They also stabilize sand dunes and improve plant cover over arid lands. This in turns prevents or delays desertification. However, at their slow rate of growth and almost constant exposure to drought stress, halophytes may not have the ability to support desert ecosystems efficiently. By finding alternative and more natural methods to improve the growth of plants, farmers may consider halting the use of traditional methods of improving plant growth and shift to more sustainable and natural ways. By closely understanding their growth and developing ways to increase it, they can support the desert ecosystems more efficiently. It is possible to isolate beneficial plant growth promoting bacteria from different parts of these halophytes (soils and root) and then reintroduce them to the plants. Inoculation can improve some or all the growth parameters of plants depending on many factors such as the microbial activity , microbial compatibility, and age of plants.

For future studies, it is advised to do the microbial compatibility test on bacterial strains before selecting them for inoculation. Applying the microbial treatment at early plant growth stage may also result in better observations and assessments.

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Appendices

Appendix I

(COMPOSITION OF MEDIA)

Inorganic salt-starch agar (starch nitrate agar) (SNA) (Kuester, 1959)

Soluble starch	10 g
Potassium nitrate	2 g
Di-potassium hydrogen phosphate	1 g
Magnesium sulfate	0.5 g
Sodium chloride	0.5 g
Calcium carbonate	3 g
Ferrous sulfate	0.01 g
*Trace salt solution	1 mL
Cycloheximide (Sigma)	50 μg mL-1
Nystatin (Sigma)	50 μg mL-1
Distilled water	1 L
Agar	20 g

*Trace salt solution (*Pridham et al.,* 1957) composed of: 0.1 mg liter-1 of each of the following salts: ferrous sulfate, magnesium chloride, copper sulfate and zinc sulfate.

Glucose peptone broth (GPB) (Di Menna, 1957)

Glucose (Sigma)	10 g
Peptone (Sigma)	5 g
L-Tryptophan (L-TRP) (Sigma)	5 mL of 5%
Distilled water	1 L

Moeller's decarboxylase agar medium (MDAM) (Arena & Manca de Nadra, 2001)

Peptone (Sigma)	5 g
Yeast extract (Sigma)	3 g
Glucose (Sigma)	1 g
Pyridoxal-5-phosphate (Sigma)	0.03 g
Manganese sulfate	0.03 g
Phenol red (pH dye indicator) (Sigma)	0.02 g
L-arginine-monohydrochloride (Sigma)	2.00 g
Distilled water	1 L
Agar	20 g

Nutrient agar

Beef extract (Sigma)	1 g
Peptone (Sigma)	5 g
Yeast extract (Sigma)	2 g
Sodium chloride	5 g
Distilled water	1 L
Agar	20 g

Medium for phosphorus solubilization (Pikovskaya agar medium (PVK) (Pikovskaya, 1948)

Glucose	10.0 g
Calcium Phosphate $Ca_3(PO4)_2$	5.0 g
Ammonium sulfate $(NH4)_2SO_4$	0.5 g
Sodium Chloride	0.2 g
Magnesium sulfate	0.1 g
Potassium chloride	0.2 g
Yeast Extract	0.5 g
Manganese sulfate	0.002 g
Iron sulfate	0.002 g
Bromophenol blue	5 ml of 0.5% dissolved in 100% ethyl alcohol
Distilled water	1000 mL
Agar	20 g

Appendix II

Table 2: List and properties of selected PGPB isolated from *Salsola imbricata* and *Zygophyllum mandavillei* plants.

Serial number	Code	Species	Source	Growth media	Shape	Gram stain	PGPR
1	S.19	*Salsola imbricata*	Soil	NA	Bacillus	-ve	Polyamine
2	S.30root	*Salsola imbricata*	Roots	NA	Bacillus	-ve	Polyamine
3	S.21root	*Salsola imbricata*	Roots	NA	Bacillus	-ve	Polyamine
4	S.15	*Salsola imbricata*	Soil	NA	Bacillus	-ve	Polyamine
5	Z.12	*Zygophyllum mandavillie*	Soil	NA	Bacillus	-ve	Polyamine
6	S.12root	*Salsola imbricata*	Roots	NA	cocci	-ve	Phosphorus
7	S.29root	*Salsola imbricata*	Roots	NA	cocci	-ve	Phosphorus
8	S.27root	*Salsola imbricata*	Roots	NA	cocci	+ve	Phosphorus
9	S.22root	*Salsola imbricata*	Roots	NA	cocci	-ve	Phosphorus
10	S.11root	*Salsola imbricata*	Roots	NA	Bacillus	-ve	Phosphorus
11	Z.7root	*Zygophyllum mandavillie*	Roots	NA	Bacillus	-ve	Auxin
12	Z.32root	*Zygophyllum mandavillie*	Roots	NA	Bacillus	-ve	Auxin
13	S.2	*Salsola imbricata*	Soil	NA	cocci	-ve	Auxin
14	Z.5root	*Zygophyllum mandavillie*	Roots	NA	Bacillus	-ve	Auxin
15	S.26root	*Salsola imbricata*	Roots	NA	Bacillus	-ve	Auxin