Enhancement of *Nitraria retusa* Growth by Rhizospheric Microbial Inoculum

Z. Baroon
A. Yateem*
T. Al-Surrayai

Kuwait Institute for Scientific Research
Biotechnology Dept., PO Box 24885
13109 Safat, Kuwait
*corresponding author
ayateem@safat.kisr.edu.kw

ABSTRACT
Native desert vegetation in Kuwait has been severely depleted due to both natural and anthropogenic factors and are facing the danger of extinction. Symbiotic rhizospheric microflora influences the growth of plant communities in different ecosystems. The objective of this study is to emphasize the enhancing effect of rhizospheric microbial inoculum on the growth of native desert plants. A shed house experiment was conducted using *Nitraria retusa* which was selected on the basis of its importance and potential for the revegetation of desert flora. The plant was propagated in three different soil treatments: soil with added rhizospheric inoculum (SI), soil with added amendment (SP) and soil with added rhizospheric inoculum and amendment (SIP). The growth performance of *N. retusa* in terms of shoot height and number of leaves was monitored on a monthly basis during 120 d experimental duration and compared with control soil treatment (SC) which was soil without any additions. The results clearly demonstrated the enhancing effect of rhizospheric microbial inoculum when combined with fertilizers in soil amended treatment (SIP) on the growth of *N. retusa*. Additionally, *N. retusa* in the inoculated treatment (SI) maintained a high survival rate during the experiment compared to other treatments.
Keywords: Native desert plant; symbiotic microflora; growth performance.

Introduction

Arid regions are characterized by a set of climatic conditions which include a long dry and hot summer, with scarce, erratic, but torrential rainfalls. This climate, together with anthropogenic degrading activities, such as overgrazing, unregulated desert management, off-road driving, camping, and industrial practices are a major threat to the sustainability of the desert ecosystem in the state of Kuwait (AboEl-Nil, 1997; Brown, 2003; Khalaf, 1989). More seriously, aggressive damage was inflicted on the natural environment during the Iraqi invasion and the Gulf War (Karrar et al., 1991). As a result of these natural and human factors, the native desert vegetation of Kuwait has been severely depleted and is facing the danger of extinction (Omar, 1991; Omar and Zaman, 1995; Omar et al., 2001; Omar et al., 2006; Taha et al., 1988; Zaman, 1997).

Conservation of native plants through biotechnological methods can play an important role in the rehabilitation of degraded desert. In order to maintain optimum soil conditions and allow maximum sustainable grazing capacity in Kuwait, it is vital to conserve and, where necessary, re-establish the natural vegetation, adapted to the local environmental conditions.

Soil microbial communities are immersed in a framework of interactions that affect plant stability and soil quality especially in the rhizosphere zone. In the rhizosphere the symbiotic soil micro and macro-organisms such as bacteria, protozoa, nematodes, mites, small insects, and fungi (primarily vesicular-arbuscular mycorrhizae) interact as a complex web that is critical for energy and nutrient flow in plants ecosystems (Manske, 2002). Strategic and applied research has demonstrated that rhizospheric microbial activities are considered to be key tools that enhance sustainable and environmentally friendly practices between the soil and host plant (Barea, 2000; Barea et al., 2002; Bowen and Rovira, 1999; Glick, 1995; Gryndler, 2000; Kennedy, 1998; Linderman, 1992; Lynch, 1990; Toal et al., 2000). In addition, rhizospheric mycorrhizal fungi can enhance revegetation and help plants to thrive in arid conditions by increasing soil fertility as they alter nutrient cycling and supply (Nelson and Safir, 1982; Requena et al., 2001; Toro et al., 1997), improve soil aggregation in eroded soils (Caravaca et al., 2002), and reduce water stress (Auge', 2001). The rhizosphere is an important site of microbial activity in desert soils since it provides ample carbon substrate in an otherwise organic matter poor arid soil. Therefore, the rhizosphere effect is more pronounced in desert soil both qualitatively and quantitatively than other soils (Bhatnagar and Bhatnagar, 2005; Buyanovsky et al., 1982; Yateem, et al., 2000).

In recent years there has been a renewed interest in the use of rhizospheric and soil microflora which when applied to seeds, roots or tubers are able to colonize plant roots and stimulate plant growth and crop yield (Höflich et al., 1994; Jeon et al., 2003; Omar and Abdalla, 1994). The mechanisms of plant growth promotion by non-pathogenic, plant associated microorganisms have not been completely elucidated, but the important mechanisms include direct phytohormonal action, plant disease suppression and the enhancement of other plant beneficial microorganisms (Jeon et al., 2003). To date many studies on the inoculation of plant growth promoting rhizosphere microorganisms have focused on some economically important crops, while

only few considered wild flora as a research target (Bashan, 1998; Glick, 1995; Jeon et al., 2003).

The intent of this study is to emphasize the enhancing effect of rhizospheric microbial inoculum on the growth of native desert plants in Kuwait. Shed-house experiments were conducted to compare the growth performance of *N. retusa*, a native desert plant species, using different soil treatments.

Materials and Methods

Climate and Soil Characteristics

Kuwait is an arid country located in the northwestern corner of the Arabian Gulf. Its climate is characterized by a very hot summer and mild winter with limited rainfall. The desert soil is mostly sandy in nature, and it is alkaline soil with limited organic matter (<1%), having very low content of plant nutrients and high amounts of calcareous materials. Soil water holding capacity is low (7%) with high infiltration rate (50-100 cm/h) and high evaporation (3000 mm) (Abdal et al., 2002).

Plant Selection

A perennial shrub, *Nitraria retusa* (local name: Ghardaq) was selected due to its importance as native grazing rangelands plant community and its ability to tolerate extreme adverse conditions such as drought and salinity. Additionally, this plant species was classified among forty important native plant species selected for the propagation and seed production at the Range and Animal Development Center, Al-Jouf, Saudi Arabia (ICARDA-APRP, 2002). In Kuwait, *N. retusa* is under severe pressure from grazing animals and harsh climatic conditions (Sudhersan et al., 2003).

N. retusa belongs to the botanical family *Zygophyllaceae*. It grows along shallow sand hummocks on saline ground near the coastal areas. It is an important sand controller that is salt-tolerant and drought-resistant (Shaltout et al., 2003). It bears fleshy red fruits that are tasty produce a refreshing juice. Many wildlife forms feed on the fruits of this plant. The natural propagation of this species is through seeds.

Soil Sampling and Analysis

The starter soil was collected from an undisturbed area containing native vegetation including *N. retusa* selected for the study. Approximately, 40 kg of soil were collected from the area of the selected plant species site to a 30 cm depth, placed in plastic bags and transferred promptly to the laboratory. Representative rhizosphere soil samples 20-30 cm deep associated with the roots of the selected plant were collected using either auger or shovel according to the methodology of rhizosphere soil collection and sampling as described by Wollum (1982).

Microbiological analysis was carried out to determine the existing rhizospheric microbial populations in the collected rhizosphere soil samples. Total bacterial and fungal counts were determined using spreading technique standard method as described by Lorch et al. (1995). The counts were expressed as colony forming unit (CFU)/ g of soil. Nutrient agar (NA) medium (Difco) was used for bacterial enumeration of the analyzed samples. Plates were incubated aerobically at 37°C for 24-48 hrs, while potato dextrose agar (PDA) medium (Difco) was used to determine fungal counts in the tested samples and plates were incubated aerobically at 30°C for 3-4 days.

Seed Collection and Germination

Fresh seeds of *N. retusa* were collected from different locations that represent the selected desert plant natural habitats in Kuwait. The selected locations are Agriculture Research Station in Sulaibiya, Shegayah, Sabhan and Doha areas. The collected seeds were cleaned and stored in dry paper bags at room temperature. Only seeds that contained healthy and filled embryo were selected for the study.

The germination procedure was carried out at the seed germination laboratory in Agriculture and Aridland Department in Kuwait Institute for Scientific Research. The germination process was carried out at room temperature (25°C) using disposable Petri dishes lined with Whatmann filter paper (No.1, 9 cm in diameter). The filter paper was moistened with 5 ml of distilled water prior to sowing. After sowing, Petri dishes were covered and sealed to avoid evaporation (Zaman et al., 2006). Four replicates each with 25 seeds per plant were maintained. The seed was considered germinated when the radical protruded to a length of at least 2 mm. The germination process was recorded daily until no seeds germinated for three consecutive days.

Starter Rhizospheric Inoculum

Ectomycorrhizal fungal inoculum was prepared under laboratory conditions. The roots, with the adhering rhizosphere soil, were cut into 1 to 2 cm long segments, and then 2.5 g of segmented roots of the selected plant with adhering soil were placed in 100 ml Erlenmeyer flasks containing 25 ml of modified Melin-Norkrans medium. The medium constituted of the following (g/L): 0.5 g of $(NH_4)_2HPO_4$, 0.3 g of KH_2PO_4, 0.14 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $CaCl_2 \cdot 6H_2O$, 0.025 g of NaCl, 10 g of D-glucose, 0.001 g of glutamic acid, and 0.0001 g of thiamine. The pH of the medium was adjusted to 5.5 with 1 M HCl. Flasks were incubated at 28°C for 17 d in absence of light and were shaken manually every 2 d to allow aeration.

Bacterial inoculum was similarly prepared using plate count (PC) broth medium (Difco). An amount of 20 g of segmented roots of *N. retusa* with adhering soil were placed in 1000 ml Erlenmeyer flasks containing 500 ml of plate count broth medium. Flasks were incubated at 30°C for 8 d in an orbital shaker at 150 rpm.

Experimental Design

Soil treatments were prepared to assess the effect of

rhizospheric microbial inoculum on the growth of the selected native plants. The experiment was carried out in a shed house using a 4 × 1 × 10 factorial design as complete randomized block consisting of four treatments including the control, plant species and ten replicates in each treatment. Soil treatments were as follows:
Soil with added inoculum (SI).
Soil with added amendment (SP)
Soil with added inoculum and amendment (SIP)
Control soil with no inoculum or added amendment (SC)

Germinated seedlings of *N. retusa* were planted in ready-to-use 42 mm Jiffy-7 pellets (Jiffy International AS, Norway) made from sphagnum peat, lime and low ammonium fertilizer. Prior to use, Jiffy pellets were moistened with water and left overnight to allow expansion. After planting the germinated seeds, the pellets were sprayed with water once every 2-3 d. As the seeds exhibited successful growth (approximately 3 to 4 wks), the pellets were carefully planted in the center of 18 × 20-cm round plastic pots (one seedling per pot) filled with 1kg soil. The inoculum was placed 5 cm below the soil surface and near the roots at the time the seedlings were transplanted in the pots of (SI) and (SIP) treatments. Each seedling received rhizospheric inoculum consisting of 100 ml of naturally abundant rhizospheric bacterial inoculum with densities of 10^8 CFU/ml, and 5 ml of ectomycorrhizal fungal inoculum. Soil amendment that contained N:P:K 21:21:21 with trace elements (Albatros Universal, Netherlands), was added to the seedlings that were planted in the treatments amended with fertilizer, (SP) and (SIP), at a rate of 0.008 M per pot. Fresh irrigation water was applied once every 2 d to maintain proper moisture. The experimental duration was 120 d which represents the estimated time needed by the seedlings to grow and develop adequate lateral roots. Plant growth performance was monitored on a monthly basis by measuring plant height and recording leaf number. Observations of plant survival in different soil treatments were reported.

Statistical analysis

The collected data summarizing the plants growth in different treatments and replica were statistically analyzed by applying 1-way analysis of variance (ANOVA) test for significance at $P \leq 0.05$.

Results

Enumeration of Rhizosphere Microbial Populations

Microbiological examination of rhizospheric soil samples around the roots of the selected native plants indicated numbers of naturally indigenous bacterial and fungal populations in the arid soil environment. The numbers of the detected viable aerobic bacterial counts ranged from 10^4 to 10^5 CFU/g of soil, while, the detected fungal counts ranged from 10^2 to 10^4 CFU/g of soil. There was a slight variation in the detected bacterial and fungal populations between the different sampling locations. This was attributed to the nature of soil and abundance of naturally existing plants.

Plant Growth

In the first month, there was no significant difference in the growth performance of *N. retusa* as the plant exhibited similar growth in all four soil treatments including the control. However, the difference was more distinct in the months following transplanting as illustrated in Figs. 2, (a, b, c, d). Inoculum addition in SI treatment had no significant effect on *N. retusa* shoot growth, especially when compared to the control treatment during the transplanting period. However, in SIP treatment, where the rhizospheric microbial inoculum was combined with soil amendments, effective growth parameters with respect to shoot height and leaf numbers were observed. Upon comparing SIP treatment with SP treatment, which only differs in the added rhizospheric inoculum, it was noticed that the growth performance was significantly higher in SIP compared to SP treatment. Additionally, the survival of *N. retusa* after four months varied with the different treatments. While the plant maintained a 100% survival in SI soil treatment during the experiment duration, it was noticed that the survival rate decreased with the experiment duration in all other soil treatments from 100% to 80% in SIP treatment and 70% in SP and SC treatments.

Discussion

Populations of aerobic bacteria in deserts were literally reported to vary from <10 to 10^7, while fungal populations as viable propagules were lower due to the low levels of fungal sporulation in arid regions (Stutz and Morton, 1996), and ranged from nil to 10^3 (Bhatnagar and Bhatnagar, 2005). Desert soil samples of Kuwait revealed that the viable bacterial and fungal counts were within the reported world values (Yateem et al., 2000). However, microflora numbers and community structure in the Kuwaiti desert varied with seasonal changes in temperature. Additionally, the Gulf environmental crises and oil spill locations had severely affected the rhizosphere environment of desert plants (Radwan et al., 1998; Radwan et al., 2000; Radwan et al., 2004; Radwan, 2008; Yateem et al., 2007; Yateem et al., 2008). The detected microbial counts in the rhizosphere soil samples of the tested plant species suggest the presence of weakened rhizospheric root symbiosis which is contingent on active rhizospheric microbial communities in the soil (Allen and Allen, 1990).

In this study, the effect of rhizospheric microbial inoculum on *N. retusa* growth was clearly demonstrated in SIP treatment in which the inoculum was combined with soil amendment and fertilizer. However, the addition of microbial inoculum alone did not produce a pronounced effect on the growth performance of *N. retusa* in terms of shoot height and leaf number in treatment SI but rather maintained a constant growth of a healthy plant with 100% survival rate. This finding is very important as the revegetation of native desert plants depend basically on their survival in their environment and the rhizospheric microbial inoculum clearly facilitated this issue.

The microbial inoculum in this study was prepared from the rhizospheric microbial communities associated with *N. retusa* that was added after being enriched as a consortium. The rhizosphere soil contains a balanced spectrum of microorganisms that occupies 5-20% of the root surface and most colonization occurs in the area of maximum root exudation (Suslow, 1982). However, at an early stage of root development it does seem possible to manipulate this balance, in order to promote plant growth by inoculating with selected rhizospheric microorganisms instead of microbial consortium (Höflich et al., 1994). In this context, the symbiotic rhizospheric strains recovered from the experimental selected desert areas can be considered 'ecotypes' as nominated by Jeffries and Barea (2000), which are physiologically and genetically adapted to the environment of the desertification-threatened ecosystems.

Conclusions

This shed house experiment examined the effect of rhizospheric microbial inoculum as a growth enhancer of *N. retusa*. *N. retusa* is one of the most important native plants for revegetation purposes in the desert of Kuwait. Regardless of its limited effect on the growth of *N. retusa* shoots, the application of rhizospheric microbial inocula clearly demonstrated a positive effect on the plant's survival.

According to the results of this study it was concluded that the introduction of symbiotically indigenous rhizospheric-plant associations could be a successful biotechnological tool to promote the recovery of degraded ecosystems. The findings of this study did not only broaden the knowledge of rhizosphere microbial interaction with the selected plant but also may contribute to the issue of a better implementation of rehabilitation plans. Prospectively, further field investigations are strongly recommended to be carried out to support and prove this conclusion.

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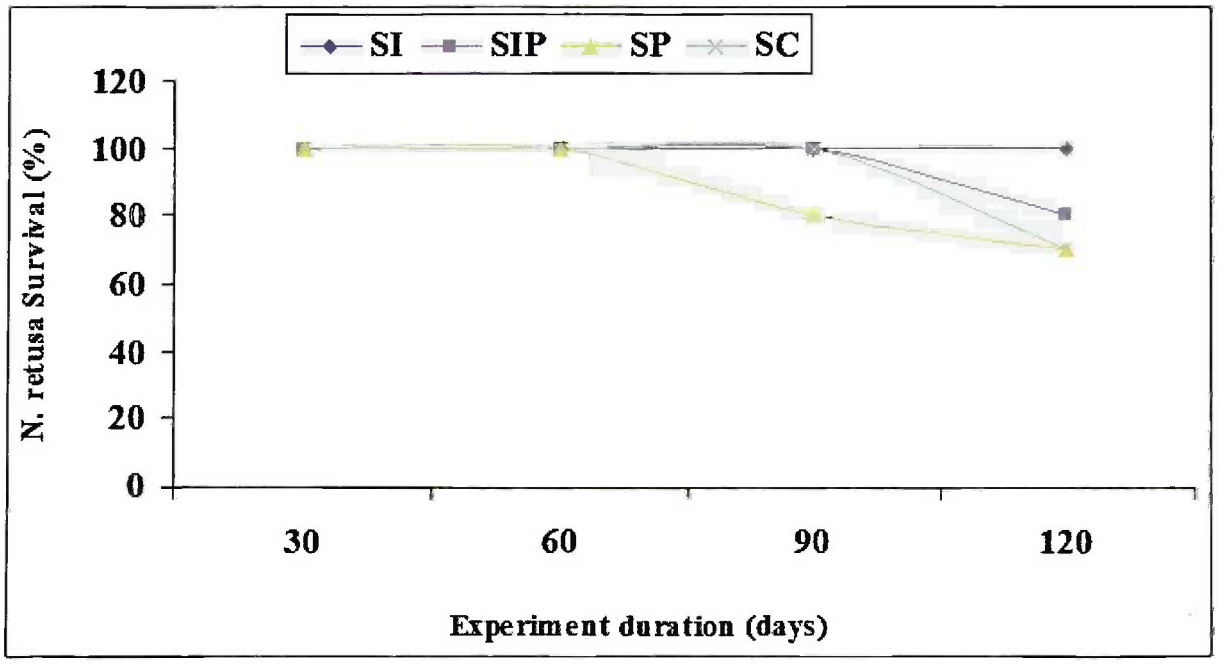
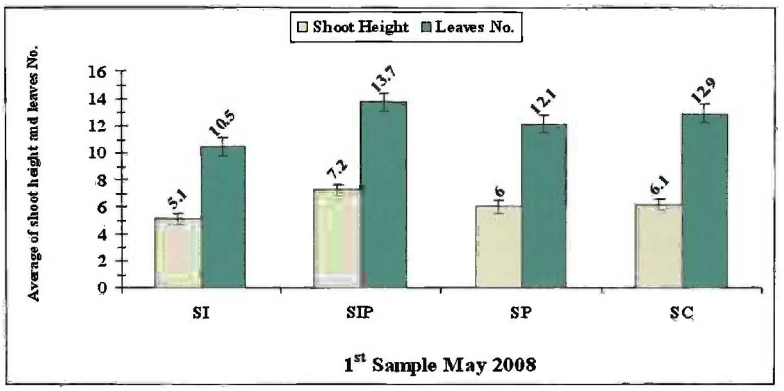
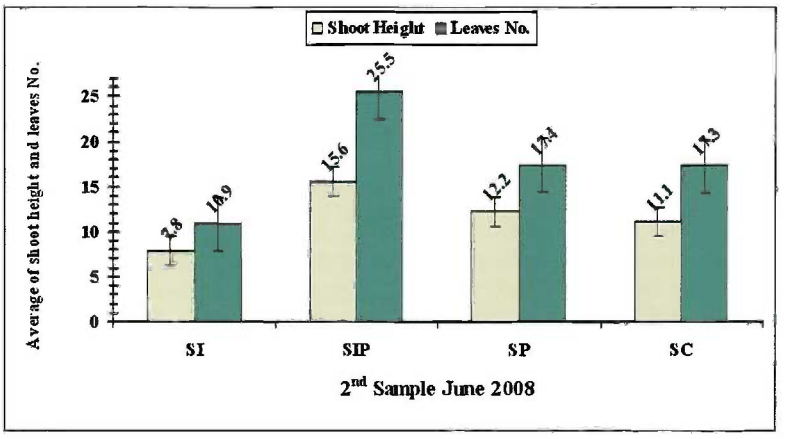
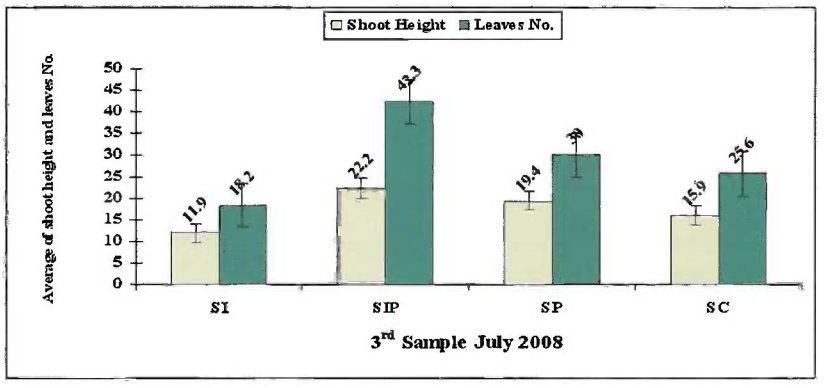


Figure 1. Survival rate of *N. retusa* (%) in soil treatments during 120 d.







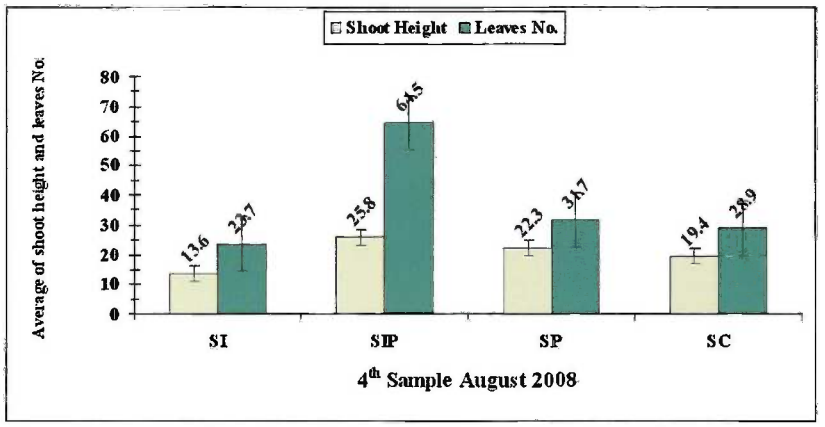


Figure 2. Growth performance of *N. retusa* during the 120 d: (a) 30 d, (b) 60 d, (c) 90 d, and (d) 120 d.
SI = Soil with added inoculum
SIP = Soil with added amendment
SP = Soil with added inoculum and amendment
SC = Control soil with no inoculum or added amendment