DOCTOR OF PHILOSOPHY

Effect of environmental stresses and growing medium amendment with ‘Zander’ on growth of Acacia saligna under saline conditions

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Award date:
2011

Awarding institution:
Coventry University

Effect of environmental stresses and growing medium amendment with 'Zander' on growth of *Acacia saligna* under saline conditions

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A thesis submitted in partial fulfilment of the University's requirements for the Degree of Doctor of Philosophy

MAY 2011

Coventry University

School of Business, Environment and Society

Zander Organic

Abstract

In Libya salinization of land and ground water is a key problem. *A. saligna* is one species that offers potential for growth under these conditions. Experiments were undertaken to accelerate the germination of *Acacia* seeds, and various pre-treatment methods were assayed. Boiling water treatment, mechanical scarification and acid improved germination and germination rate. Sowing *A. saligna* seeds at 30 mm depth gave greatest seedling growth from large and medium seeds whereas 20 mm was more suitable for small seeds. This study aimed to improve the establishment of *Acacia saligna* irrigated with saline water, plants were grown for seven weeks under greenhouse or field conditions in (Libya) either sand or soil salinized with varying applications of NaCl. Irrigation with NaCl significantly decreased plant survival and growth and concentration of Ca, Na, K, Fe and P ions in plants with 0.5 M or 1.0 M NaCl. The experiments were repeated using a naturally occurring soil amendment called 'Zander'. Seeds of *A. saligna* were grown for seven weeks in both greenhouse and field trials as before but with the addition of Zander and NaCl. Zander improved plant survival and growth with salinity and increased the elements in plants (Ca, Na, K, Fe and P). Field experiments were conducted to assess the effects of saline irrigation with 1.0 M NaCl and extra water added to 0% or 10% Zander on survival and growth, consequently, seedling growth significantly decreased with increase in soil salinity. Survival and growth increased with increase in extra water. The additional irrigation water caused an increase in the uptake of Ca^{++} and increased the Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio. Zander did not appear to reduce net uptake of Na^{+} and its transport to shoot tissues. Mg^{++}, P, K^{+} and Ca^{++} content significantly decreased in plants in response to salinity. Possible mechanisms to avoid Na^{+} toxicity in *A. saligna* in response to salinity included increasing the supply of Ca^{++}. Extra Ca^{++} applied into the medium with and without salt increased survival and growth even in the absence of Zander. Calcium increased uptake of Ca^{++} and increased Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio.

Acknowledgments

First of all I thank Allah, for helping me achieving this work. Without his guidance, this work would never have been accomplished. I am grateful to many people for help, both direct and indirect with the research.

Then I would like to thank Prof. Philip J. Harris for his supervision, encouragement and support throughout the entire course of study, and for his critical guidance during the preparation of this manuscript and for the use of facilities of the Faculty of Business, Environment and Society.

I would also like to thank my other main supervisors; Dr James Bennett and Dr Mark Bateman of Coventry University for their invaluable assistance and constant encouragement during my work, particularly when progress was difficult. Many other people also assisted me during my fieldwork and writing up. Laboratory technicians Neil Thompson and Richard Collins for help in laboratory and providing material. I wish to thank all postgraduate students and research associates for creating a friendly environment.

The Libyan High Education and Al Fateh University are acknowledged for providing me with a grant to carry out this research.

I am sincerely grateful to my mother and my family, relatives and all friends for their assistance in many ways. I am grateful to my husband, for his encouragement, understanding and tolerance during the course of the study.

Finally, I am very much indebted to my daughters who stayed away from me in Libya and my son who accompanied during my study. It would be unimaginable to complete this study without their tremendous patience, understanding and support.

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List of Abbreviations

ANOVA	Analysis of Variance
Ca^{++}	Calcium
cm	Centimetre
D	Day
D.W	Distilled water
DW	Dry weight
EC	Electrical conductivity
Fe^{++}	Iron
FW	Fresh weight
K^{+}	Potassium
Na^{+}	Sodium
Mg^{++}	Magnesium
P	Phosphorus
Cl^{-}	Chloride
$1/t_{50}$	The reciprocal of the time in days taken to complete 50% of final germination
Z	Zander

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Chapter 1

Introduction

1.1 Introduction

Large areas of globally are suffering from serious degradation such as soil salinization and sodicity (Karen 1994). Secondary salinization is widely considered as a key process leading to degradation and desertification of the world's dry lands (Thomas and Middleton 1993), water logging and the combination of these factors results from soil salinization and sodicity, which are the most common forms of degradation (Szabolcs 1994). The magnitude of these factors is especially severe in arid and semi-arid regions of the world (Singh and Singh 1995), and they result in loss of soil fertility and vegetation cover, and therefore increase environmental problems (Singh and Singh 1995). It has been estimated that about one billion hectares of the world's land is affected by salt. Of this 60% is cultivated. In a fraction of these soils, salt accumulation in the soil profile can be attributed to natural processes, but in the majority of cases it is brought about by human intervention due to introduction of irrigation, use of saline water or due to other developmental works leading ultimately to accumulation of salts (Goyal, Sharma, and Rains 2003). Salinity is increasing at an alarming rate and has already converted vast fertile lands into bare degraded soils. The impact of salinity on the economic exploitation of land for agriculture and forestry is very severe (Singh and Singh 1995). Some harmful impacts of salinity on agricultural production, soil physiochemical properties and associated ecological balance of the area have been listed by Chhabra (1996) and include low agricultural production and increased soil erosion.

1.2 Environmental problems

Saline soils are characterized by excessive concentration of soluble salts, especially those of sulphates and chlorides of sodium, calcium and magnesium, and small quantities of carbonates and bicarbonates (FAO 1997). Libya has only a limited area of land available for agriculture and forestry and nearly 100,000 ha of land are being affected by salinity and water logging every year. The need for more food for the increasing population demands the use of proper reclamatory measures to get this land back into cultivation.

Salinity is a major abiotic stress that limits plant growth and productivity and can be simply defined as the relative proportion of salt in a solution. Generally, salinity is defined as the presence of excessive amounts of soluble salts that hinder or affect the normal functions of plant growth (Ashraf and Harris 2002). Salinity is considered to be harmful when soil EC readings are greater than 4 dS m^{-1} and of little concern at EC less than 2 dS m^{-1}. There are several key physiological features that are common in salt tolerant plants. Salinity usually appears on the soil surface just after spring, soluble salts most commonly present are the chlorides and sulphates of sodium, calcium and magnesium. Nitrates may be present in appreciable quantities leading to high salt uptake with no damage within shoot organs, and is sometimes accompanied by salt excretion from leaves. Sodium and chloride are the most dominant ions, particularly in highly saline soils, although calcium and magnesium are usually present in sufficient quantities to meet the notional needs of crops (FAO 1985). The ability of plants to survive salinity is important for natural distribution of plant species and agriculture (Flowers and Yeo 1986). The interaction of ions in plants and soil also plays a significant role in the

salinity tolerance of plants and a clearer understanding of the dynamics of this process would enhance our general knowledge of salinity tolerance.

Libya, like many other countries, faces this problem and according to some estimates about 50-80% of agricultural land is affected by salt. The reduction in productivity of soils affected by salinity is about 30% (El-Lakany and Luard 1986), threatening the livelihoods of poor farmers and having a significant negative impact on food production as a whole. Moreover, the Libyan Government has spent large sums on reclamation, mainly on drainage projects (more than US$ 30 million annually) to solve salinity problems in irrigated areas, but the annual average net income from crops grown with drainage is more limited than for those grown without drainage. As a result of the low precipitation in Libya, groundwater resources have been used in the development of agriculture (Ben-Mahmoud, Mansur, and Al Gomatl 2000). The expanding economy and growing population along the coastal strip is creating an increasing demand for groundwater resources. Hence, the traditional groundwater resources are become increasingly at risk through intensive use, which in turn is resulting in saline intrusion into the coastal aquifer. Libya suffers from severe salinity problems. This salinization is mainly due to low precipitation, high surface evaporation, poor drainage system with 98% of the cultivated land under irrigation, and rising water table (less than 1 m below the soil surface). Coastal areas in Libya may even be more directly affected by salinization due to the increased penetration of sea water into the groundwater aquifers (Plate 1.1).

Research in Libya has focused on the utilization of saline water for agriculture for over half a century. The problem of salinity may be addressed by using technical (water and

soil management) and biological approaches, e.g. tree plantation. Reclamation of saline areas by physical or chemical means is not only expensive but it may also raise environmental concerns. Aydarov (1996) reported that current practices used for reclamation of saline soil ignore the natural environment and are often accompanied by deterioration in local ecology. Therefore, traditional engineering approaches to salinity problems are no longer adequate and suitable. Plate 1.1 show saline water irrigation effect for agriculture in Libya.

Plate 1.1 Mapping of Natural Resource saline soils in Libya Arab Jamahiriya (LIB 2004)

The best strategy to overcome salinity may possibly be to grow woody vegetation that can be economical and beneficial to local communities and also protect the environment. *Acacia saligna* has attracted considerable interest as a multipurpose tree in a wide range of climatic zone.

Plate 1.2: *Acacia saligna* (*A. cyanophylla*) in Libya (17.10.2008)

Acacia saligna genus *Acacia* within the family Leguminoseae and sub-family Mimosidae which display considerable drought tolerance and tolerance of waterlogged, alkaline or saline soils, and which fix atmospheric nitrogen through a symbiotic relationship with bacteria of the *Rhizobium* group. Many species of leguminous shrubs, particularly *Acacia saligna* have proved to be useful multipurpose shrubs in North Africa and Libya (El-Lakany 1987). *Acacia saligna* is the most successful of the Australian *Acacia* species growing in Libya owing to its tolerance of drought, ability to grow on poor soil, high production of biomass and high nutritive value (Plate 1.2). Sodium is the most common salt in saline soils. This study had been carried out using sodium chloride to evaluate the tolerance of *A. saligna* which is the most common introduced legume tree in Libya.

1.3 Characteristics and applications of Zander

Zander is a fibre amended mineral material, originating in lacustrine sediments derived from naturally occurring organic deposits laid down over several thousand years in a sustainable system of anaerobic decomposition associated with fresh water systems in many parts of the world. When used in relatively small amounts it can have a lasting effect in areas of the world affected by overgrazing, over-cultivation and desert encroachment, and has little environmental inpact (Zander Corporation 2007). Zander is extracted from freshwater lake sediments in many parts of the word (Zander Corporation 2007). It is a fine, moist, black, humic substance with a high concentration of organic compounds, minerals and available plant nutrients. There have been a number of recent tests of the potential of Zander to ameliorate the effect of drought, salinity, and soil contaminated with oil or heavy metals (Zander Corporation 2007).

1.3 Research problem

There are limited high quality water resources in Libya, and there has been over exploitation of ground water resources in agricultural, industrial and domestic sectors. This over exploitation has caused deterioration in water quality and a decrease in its availability. This has led to consideration of how to use the remaining low quality water for irrigation, irrigation water management, type of trees to be irrigated, soil and water salinity monitoring, and leaching of salts. The increase in human population makes it imperative that more resources be assigned to food production systems, even in areas with limited water resources and marginal land with saline soils. A single approach will not provide all the necessary techniques to address this problem and it will require landscape manipulation techniques to improve and sustain the productivity of these soils. This will lead to the good quality water being saved for domestic use, irrigation of sensitive crops and industry. *A. saligna* plays an important role as fodder for livestock and firewood for the inhabitants of desert and semi-arid areas of Libya. Methods of planting *A. saligna* are reasonably well understood but how plants should subsequently be managed under the environmental conditions of Northern Libya is less well understood. The main problem for range programmers is the poor germination of seeds. *A. saligna* will not germinate easily when placed under conditions of drought or severe salinity. Consequently, there is a pressing need to investigate in which saline water concentrations *A. saligna* could be used. Zander which is naturally occurring mineral and has the following properties 1- Provide mineral nutrients and improve cation exchange. 2- Improve water holding capacity. 3- Contains nutrient required for sustained growth plants. 4- Enhance soil microbial biomass. 5- Protect plants from abiotic soils stresses such as salinity. These qualities have made Zander a potential bioremediating agent for salinity.

1.5 Research objectives

The aim of this research was to improve the establishment of *Acacia saligna* in arid and semi arid soils under saline conditions using a growing medium amendment (Zander).

The specific objectives were:

1- To determine the effect of salinity stress on establishment and growth of *Acacia saligna.*

2- To determine the extent to which Zander can ameliorate the effect of salt on *Acacia salign*a.

3- To evaluate the optimum methods for *Acacia saligna* establishment under Libyan field conditions and make recommendations for improved nursery practices.

4- To elucidate the physiological mechanisms underlying the interactive affects of growing medium and saline stress on *Acacia saligna*.

1.6 Structure of the thesis

This thesis comprises eight chapters, chapter 1 : 1- provides an overview of the potential of *Acacia* species as useful legumes, 2- the effect of salinity on plant growth and environmental problems 3- Zander characteristics 4- research problem and research objectives. Chapter 2 gives a general literature review including sources of salinity, effect of salinity on soil, effect of salinity on plants, toxic effect, osmotic effect, plant growth and yield as a measure of salinity, physiological responses of plants to salt stress, organic amendment, Zander, genus *Acacia*, seed factors and planting *Acacia saligna*. Chapter 3 reports the development of efficient methods for removing hard seed dormancy, including soaking in boiling water, cutting, sulphuric acid and manual scarification. The effect of seed size, pre-germination treatment and sowing depth on germination, survival and seedling growth were determined. Seeds of *A. saligna* were graded into large, medium and small sizes and their germination percentage, germination rate and establishment were recorded from different depths. Chapter 4 reports the effects of saline irrigation on establishment of *A. saligna* as a preliminary stage before testing treatments to moderate the effect of saline irrigation. Chapter 5 explores the physical and chemical characteristics of Zander and evaluates the effect of saline water irrigation on *A. saligna* grown in medium amended with different Zander levels. Chapter 6 investigates whether additional irrigation of soil with distilled water, when plants were grown without Zander could alleviate the effects of salinity in the same way that incorporation of Zander in the soil does. Chapter 7 investigates whether supplementation of saline medium with calcium at the same level as is naturally found in 30% Zander can at least partially remediate the adverse effects of salinity on plant growth. Chapter 8 provides an overall discussion and conclusion and makes recommendations of areas for further research.

Chapter 2
Literature Review

2.1 Introduction

The environmental impacts of salinity cannot be over emphasized as they have both direct and indirect economic and social impacts. The two major environmental factors that currently reduce plant productivity are drought and salinity (Serrano *et al*. 1999), and these stresses cause similar reactions in plants due to water stress. These environmental concerns affect plants more than is commonly thought. Agricultural fields become salinized mainly as a result of irrigation (Reusch *et al*. 1996). In fact, historical records show severe environmental problems can be responsible for up to a 65% reduction in yield (Serrano *et al*. 1999).

Many factors interact with salinity. For example, humidity, temperature, light, irrigation and soil fertility all alter the effect of salinity (Allen, Chambers, and Stine 1994). Salinity is common in semi-arid regions where precipitation is low; however, irrigation remains the only alternative for crop production. This has led to decreased yield in crops grown in these regions, which have often been seen as having a soil infertility problem. Responses to this have often been an increased use of fertilizers with resultant damage to soils and reduced crop yield. According to Chhabra (1996) salinity can be identified in the field by the presence of a white crust on the surface of the soil; high water table; small bushes or halophytes as the natural vegetation, patchy and stunted plant growth, wilting plants even when the soil apparently contains enough water; and short life of buildings and farm machinery due to the corrosive effect of salt. Salt tolerances are usually given in terms of the stage of plant growth over a range of electrical conductivity (EC) levels. To determine soil salinity EC, an electrical current is imposed in a glass cell

using two electrodes in a soil extract solution taken from the soil being measured (soil salinity). The units are usually given in deci Siemens per metre (dS m^{-1}). According to EC data, soils are categorized into general ranges from non-saline to very strongly saline (Table 2.1).

Table 2.1: Salinity rating and salinity units (Chhabra 1996)

Soil salinity class	Electrical conductivity (EC) dS m^{-1}	Effect on crop plants
Non-saline	< 2	Salinity effects negligible
Slightly saline	2 – 4	Yield of sensitive crops may be restricted
Moderately saline	4 – 8	Yield of many crops restricted
Strongly saline	8 – 16	Only tolerant crops
Very strongly saline	>16	Only a few very tolerant crops

The units commonly used to express salinity water and the relative conversion coefficients to electrical conductivity at 25ºC are shown in Table 2.2.

Table 2.2: Units and conversion coefficients used to express salinity (from Gucci and Tattini 1997)

Salinity	Unit	Conversion coefficient
Electrical conductivity	dS m^{-1}, mmho cm^{-1}	1
NaCl concentration	mM, mg l^{-1}	10-12
	ppm	~ 640
Total soluble salt	%	~ 0.064
Osmotic pressure	MPa	0.036

2.1.1 Distribution of salt-affected soils

Plate 2.1 shows arid and semi-arid regions. Fertile alluvial plains, river valleys, and coastal regions are considerably affected by salinity. According to Pessarakli (1994), Australia has the largest area of salt affected soil (357.3 million hectares).

Plate 2.1 Global distributions of salt-affected soils (Pessarakli 1994)

2.1.2 Sources of salinity

There are two main sources of salinity. Primary or natural sources result from weathering of minerals and the soils developed from saline parent rocks (Sposito 1989, Ashraf 1994). Climatic factors and water management may accelerate salinization in arid and semi-arid lands (ASAL). Evapotranspiration plays a very important role in the pedogenesis of saline and sodic soils. Wanjogu *et al.* (2001) reported that most of the ASAL receive less than 500 mm of rainfall annually and this, coupled with an annual potential evapotranspiration of about 200 mm, leads to salinization. Another type of salinity occurs in coastal areas subject to tides and the main cause is intrusion of saline water into rivers (Cyrus, Martin, and Reavell 1997) or aquifers (Howard and Mullings 1996).

There is also secondary salinization that is caused by human factors, mainly as a consequence of improper methods of irrigation. Poor quality water is often used for irrigation, so that eventually salt builds up in the soil unless the management of the irrigation system is such that salts are leached from the soil profile. Grainger (1986) estimated that 500,000 ha of irrigated land become desertified each year and that this only just equals the amount of newly irrigated land. Irrigation salinization is as old as irrigation agriculture itself. Nevertheless, Ohara (1997) has reported increasing levels of salinization with increasing irrigation since the 1950s, and in the Shanxi Province of China, more than one-third of the total area of irrigated land is salinized (Qiao 1995). The land area under irrigation in Kenya is estimated to be about 84,000 ha (Nigigi 2002) and according to Mugwanja, Mochiemo, and Osoro (1995) about 26,000 ha is considered salt degraded mainly due to poor irrigation management and poor drainage, especially in areas with a high ground water table. Anthropogenic salinization occurs in

arid and semi–arid areas due to water logging brought about by improper irrigation (Ponnamperuma 1984). Secondary salt affected soils can also be caused by human activities other than irrigation and include but are not limited to, the following:

(a) Deforestation: This is recognized as a major cause of salinization and alkalization of soils as a result of the effect of salt migration in both the upper and lower soil layers. The major causes for desertification are recognised as human activities and natural salinity and water logging are the types of stress.

(b) Accumulation of air-borne or water-borne salts in soils: Szabolcs (1994) has reported that chemical accumulation from industrial emissions may accumulate in the soil and if the concentration is high enough, can result in salt accumulation in the upper layer of soil. Similarly, water with considerable salt concentration such as waste water from municipalities and sludge may contaminate the upper soil layer causing salinization and alkanization (Bond 1998).

(c) Salinity in soil is caused by irrigating the crops with saline water, particularly in greenhouse and intensive farming systems.. In closed or semi-closed systems (e.g. greenhouse) salts tend to accumulate if chemicals are not removed regularly, resulting in salinity or alkalinity. In countries with intensive agriculture such as Japan and the Netherlands, this type of salinzition appears most frequently (Pessarakli 1991).

(d) Overgrazing: Szabolcs (1994) reported that this process occurs mainly in arid and semi-arid regions where the natural soil cover is poor and scarcely satisfies the fodder requirement of extensive animal husbandry. Because of overgrazing the

natural vegetation become sparse and progressive salinization develops, and sometimes the process ends up in desertification as the pasture diminishes.

2.2 Effect of salinity on soil

Mineral weathering of parent rocks, irrigation activities and soil amendment (e.g., addition of inorganic fertilizers, gypsum, composts and manures) can cause accumulation of salts in soils. Alkalization by the excessive accumulation of sodium salinity and increase in the value of the soil of the Sodium Adsorption Ratio (SAR) can also cause salinization. There is no sharp dividing line between saline and sodic soil. Salinization affects the chemical properties of soil by altering the Cation Exchange Capacity (CEC) and its physical properties. Deflocculation of clay particles causes damage to soil structure; however, as a result of the slow movement of irrigation water the hydraulic conductivity is decreased. The soil microflora, which plays an important role in the improvement of soil structure, the decomposition of organic matter, and in the nitrogen and sulphur cycles, is also affected by soil salinity (Waisel 1972, Lal and Khanna 1994). Flocculation is the process where saline water can affect soil physical properties by causing fine particles to bind together into aggregates which are favourable in terms of soil aeration, root penetration and root growth. However, sodium has the opposite effect on soils because high sodium concentrations cause soil dispersion, and clay platelet and aggregate swelling. When too many large sodium ions come between them the forces that bind clay particles together are disrupted and when this separation occurs, the clay particles expand, causing swelling and soil dispersion. Soil dispersion is unfavourable because it causes clay particles to plug soil pores resulting in reduced soil permeability. When the soil is repeatedly wetted and dried and clay dispersion occurs, it then reforms and solidifies into almost cement like soil with little or no structure.

Consequently, the three main problems caused by sodium-induced dispersion are (Pearson and Bauder 2003):

1- Reduced infiltration when soil dispersion hardens the soil and blocks water infiltration reducing plant water availability and increasing run off and soil erosion.

2- Reduced hydraulic conductivity which refers to the rate at which water flows through the soil. When water cannot pass through soil then the upper layer can become swollen and water logged. This result in anaerobic soils which can reduce plant growth and decrease organic matter decomposition rates, resulting in lowered fertility.

3- Surface crusting is produced by physical dispersion caused by impact of raindrops or irrigation water, and chemical dispersion, which depends on the ratio of salinity of the applied water. Surface crusting due to rainfall is greatly enhanced by sodium induced clay dispersion. When clay particles disperse within soil water, they plug macro-pores in surface soil by two means. First, they block avenues for water and roots to move through the soil. Second, they form a cement-like surface layer when the soil dries. The hardened upper layer, or surface crust, restricts water infiltration and plant emergence.

Other salts that contribute to salinity, such as calcium and magnesium, do not have this effect because they are smaller and tend to cluster closer to clay particles. Calcium and magnesium will generally keep soil flocculated because they compete for the same spaces as sodium to bind to clay particles. Consequently, increased amounts of calcium and magnesium can reduce the amount of sodium-induced dispersion. K^{+} concentration decreases with an increased level of Ca^{++}.

2.3 Effect of salinity on plants

Effects of salinity on plants are due to two different properties of saline media, salinization affects the chemical properties of soil by changing the cation exchange capacity and alters the physical properties, both of which are often implicated: osmotic effects and specific ion effects. The primary effect of soil salinity on plants is to retard roo growth, because it increases the energy that the plant must expend to absorb water from soil and make biochemical adjustments in order to survive. At high concentrations of salt, the external osmotic potential may be depressed below that of the cell water potential resulting in osmotic desiccation. In order to survive the plant must adjust osmotically, increasing internal solute concentrations by means of absorption of ions from the medium, synthesis of organic compounds or both together (American Society of Civil Engineers 1990).

Plants have a complex system of cells and metabolic functions, all of which are considered to suffer from salt stress. Salinity has multiple effects on plant life, and as a result plant life is disturbed either directly by the toxic effect of sodium and chloride, known as the specific ion effect, or indirectly by an osmotic effect (Ayers and Hayward 1948, Ayers 1951). The specific ion effects are due to high concentrations of sodium, which may interfere with the absorption of other nutrient elements present at much lower concentrations and serve to moderate concentrations of sodium, chloride and other ions that may be toxic to the plant. In addition to this, the plant sensitivity to salt changes during plant development. With respect to salt tolerance or salt sensitivity three broad developmental stages can be distinguished namely germination, vegetative growth and reproductive growth (American Society of Civil Engineers 1990). Salinity stress can

be caused by NaCl and can reduce the rate of germination, seedling establishment and yield of plants.

2.3.1 Toxic effect

High salt concentrations that result in the accumulation of ions in excess of their demand in the cell lead to toxicity in non-tolerant plants. Toxicity depends on the concentration of ions accumulated, but response varies between species, varieties and even among individuals of the same species. An excess of salts limits plant growth by inhibiting various metabolic and physiological functions (Fitter and Hay 1987). According to Bowen (1966) ions may cause toxicity in various ways. Ions may inhibit enzymes, act as anti metabolites, chelate with essential elements, catalyse rapid decomposition of essential metabolites, combine with cell membranes and affect their permeability, and replace the electrochemically important elements which then fail to fulfil their functions.

An example is the toxic effect of salt on membranes. Every cell is a complex system of membranes. The most important function of the cell membrane is to regulate the exchange of materials between the cell and its surroundings. Higher plant cells have such a membrane called the plasmalemma or plasma membrane. Direct salt injury leads to a breakdown of membrane structure possibly by replacing Ca^{++} by Na^{+} at the binding sites of the phospholipids of the cell membrane (Gary-Bobo 1970, Leopold and Willing 1984, Cramer, Lauchli, and Polito 1985, Zhao and Mingliang 1988, Rengel 1992, Jacoby 1994). Ca^{++} is considered as a binding agent at membrane sites and it plays a vital role in maintaining membrane integrity (Grattan and Grieve 1999). According to Leopold and Willing (1984), salt toxicity results in the production of lesions on the

membranes, generally the plasma membrane. This changes the structure and function of membrane and results in increased permeability and leakage of solutes out of cells (Bradbeer 1988, Bewley and Black 1994) Ca^{++} is also involved in biochemical and physiological processes important for growth and development of plants (Marme 1983, Marschner 1995). There is evidence that an elevated Ca^{++} concentration in the plant increases salt tolerance of plants, possibly by controlling membrane permeability and maintaining the K^+/ Na^+ ratio (Chaudhuri and Wiebe 1968, Cramer, Lauchli, and Polito 1985). At high concentration of salts the leakage of K^+ to an external solution indicates membrane damage by the toxic effect of salt (Nassery 1979, Cramer, Lauchli, and Polito 1985, Abbas, Younis, and Shukry 1991, Petruzzeli *et al.* 1992). In non-saline conditions the cytosol of higher plant cells contains a higher concentration of K^+ and lower concentration of Na^+ allowing enzymes to function optimally (Taiz and Zeiger 1991). However, the internal K^+ concentration decreases with increasing salt concentration (Nassery 1979, Ben-Hayyim, Kafkafi, and Gamore-Neumann 1987, Torres-Schumann *et al.* 1989). There are several reports which highlight the importance of K^+ nutrition under saline conditions (Bohra and Doerffing 1993, Cerda *et al.* 1995). K^+ is an important macronutrient involved in many specific functions. For this reason Na^+ which replaces K^+ cannot fulfil the plant's need for K^+ and K^+ is affected by cation exchange phenomena (Bowen 1966, Flowers and Lauchli 1983, Cerda *et al.* 1995). For example Na^+ can substitute for K^+ as an osmoticum, but not in metabolic functions (Flowers and Lauchli 1983, Leigh and Storey 1991).

In terms of their responses to potassium substitution by sodium, Marschner (1995) divided plants into four groups - (A) a high proportion of K^+ is replaced by Na^+ without any effect on plant growth, (B) a smaller portion of K^+ replaced without affecting

growth, (C) a very limited substitution takes place and (D) no substitution of K^+ is possible. He categorised plants in groups A to D as ranging from tolerant to susceptible to $Na^{+.}$ Therefore, if plants need to grow successfully in saline conditions most must be able to maintain a high ratio of K^+ to Na^+ ions in their cytoplasm (Greenway and Munns 1980).

2.3.2 Osmotic effect

Some evidence indicates that a major effect is osmotic. The low water potential of saline soil solution results in reduced water uptake by plants (Ayers 1951, Waisel 1972, O'Leary 1973, Greenway and Munns *et al* 1980). At very low soil water potentials, this condition interferes with the plant's ability to extract water from the soil and maintain turgor. Thus, in some species salt stress may resemble drought stress. However, at low or moderate salt concentration, plants adjust osmotically and maintain a potential for the influx of water (Guerrier 1996, Ghoulam, Foursy, and Fares *et al* 2002). Plant growth may be moderated under such condition but unlike drought stress, the plant is not water deficient (Shannon, *et al*. 1994). In the case of a saline medium the plant cell water potential is reduced to the water potential of the salt solution and consequently water is lost. The decreasing water potential results in a decreased osmotic potential and turgor pressure of cells, which are essential for plant cell elongation and growth (Jacoby 1994, Poljakoff-Mayber, and Lerner, 1994). The reduced water uptake caused by the salt solution results in drought and is termed physiological drought (O'Leary 1973, Karen 1994) because plants are suffering from water deficiency even though the soil contains enough water for the plant's needs.

2.3.3 Variation with age

The tolerance of plants to salinity is not a fixed characteristic but varies with the stage of growth for the same species. Abrol (1986) states the most plants are more sensitive to salinity during the germination than at the later growth stages. This has been found for alfalfa and sugar beet (Ayers and Hayward 1948). In contrast, Ungar (1974) reported that seed germination of *Hordeum vulgare* was more resistant to salt stress than later seedling stages. With rice also, the crop is more sensitive to salinity during the young seedling stage than during germination. Pearson, Ayers, and Eberhard (1966) reported that seedling survival was affected in a similar way to seed germination in comparative tests on the effects of NaCl on six arid zone species in pot trials in Pakistan. With the woody species, *Prosopis farcta*, Eshel and Waisel (1965) found both seed germination and seedling growth of populations from saline areas were less affected by NaCl than shrubs from non-saline sites. Zekri (1993) tested the salt tolerance of several citrus rootstocks during emergence and early seedling stage of development. Salinity delayed seedling emergence and reduced shoot and root biomass. No uniform trend was found between salt tolerance during emergence and that during seedling growth. Salt tolerance at emergence did not appear to be a useful indicator for the rapid screening of citrus cultivars. Johnson, Handley, and Dejong (1992) reported that selection for increased seed germination or seedling growth in saline environments did not result in improved forage yield of alfalfa under salt stress. They concluded that selection methods which include each critical growth stage may be necessary to develop cultivars with increased yield under saline conditions. A distinction has been drawn between effects on germination capacity and germination rate. For sorghum, Francois (1985) reported that selection for increased seed germination or seedling growth in saline environments did not result in improved forage yield of alfalfa under salt stress. They concluded that

selection methods that include each critical grow stage may be necessary to develop cultivars with increased forage yield under saline conditions.

2.3.4 Plant growth and yield as a measure of salinity

Plant growth and yield have been suggested as physical indicators of salinity tolerance in plants; however, evaluating field performance under saline conditions is notoriously difficult because of the variability of salinity within fields (Richards 1983) and the enormous potential for interactions with other environmental factors, ranging from gaseous pollutants, soil fertility and drainage to temperature, light flux density and transpiration water loss.

Salinity generally affects the growth of glycophytes by either ion excess in the expanded leaves or by water deficits in the expanding leaves (Greenway and Munns 1980). The main strategy for glycophytes is the control of ions in the root xylem as this restricts ion movement to the shoot (Hasegawa *et al.* 2000), but glycophytes remain passive in tolerating high levels of salinity.

One immediate response of plants to elevated salinity is a decrease in the rate of leaf expansion which consequently results in reduction of the whole plant leaf area (Orcutt and Nilsem 2000). The germination response to salinity may differ amongst plant species within the same genus. NaCl treatment reduced dry weight, leaf area and leaf number of *Brassica juncea* and *Brescia rapa* with a significant difference between the two species. This ultimately affects photosynthetic rate in plants although this alone cannot be said to account for the growth reduction caused by salinity as Munns (1993)

explains that growth is reduced before photosynthesis decreases and growth decreases more than photosynthesis does. The reduction in plant growth in saline environments is the consequence of a toxic effect on cellular and whole plant metabolism by NaCl accumulated in tissues and the reduction of water availability, consequent to the decrease in water potential in the soil (Mansour 1997).

2.3.5 Seed germination as a measure of salinity

The seed provides food reserves that the germinating seedling depends on until it become photosynthetically self-sufficient and gains access to mineral nutrient supply (Bradbeer 1988). Seed germination is a critical phase in the development of a plant and reduced seed germination percentage and a low germination rate are effects of high salinity. High levels of salinity reduce not only the final percentage of seed germination but also root and shoot growth of young seedlings, whereas low levels of salinity stimulate germination and growth of young *Acacia saligna*. Intolerance of salinity may result in physiological and biochemical disorders which prevent or delay germination or cause abnormal seedlings (Rehman *et al*. 1996, Ungar 1996). There was considerable variation in the salt tolerance among ten species of *Acacia* studied (Rehman *et al*. 1996) and this also supports this claim.

It is generally suggested that germination of seeds in saline environments is hampered because of ionic differences that cause an imbalance and water differential that creates drought condition. There are several methods by which seed germination can be improved under saline conditions but most of these methods have been tested only in laboratories rather than in field trials. It has been reported that hardening of seeds results in an increased germination percentage and germination rate (Idris and Aslam 1975).

2.4 Physiological responses of plants to salt stress

Many different features contribute to salt tolerance of shrubs, including leaf, stem and root adaptation. If plants do not obtain enough water to balance unavoidable losses they often shed their leaves. Generally, the shedding begins with the oldest leaves and progresses toward the apical meristem; small summer leaves often replace large winter leaves (Kozlowski, Kramer, and Pallardy 1991). In general, the plants have adapted to drought either by evading drought or by resisting its effects (Pratt and Gwyne 1977) declared that the deep-rooted nature of shrubs confers upon them the ability to take out moisture from greater depths in the soil. This feature allows shrubs to remain green and to produce a more dependable food source during drought. Biologically, stress can be defined as the overpowering pressure that affects the normal functions of individual life or refers to the conditions in which plants are prevented from expressing fully their genetic potential for growth, development and reproduction. Salt, cold, heat, wind, shade, nutrient deficiency, ion toxicity, air pollution and gaseous deficiency are all environmental constituents that effect plant life.

Plants can be divided into two broad groups with respect to their response to high concentration of salts: halophytes and glycophytes (Taiz and Zeiger 1991). Those adapted to saline conditions are called halophytes and those which are not adapted to saline conditions are called glycophytes. Halophytes are plants that at any stage of their life, will tolerate a critical salt concentration which will not be tolerated by glycophtes (Poljakoff-Mayber and Lerner 1994). The vegetation of saline areas adopts several different mechanisms of tolerance that enable plants to tolerate high levels of salts (Ashraf 1994). The response of vegetation to salinity is grouped as, salt avoidance, salt resistance, and salt tolerance. A variety of mechanisms contribute to salt tolerance

(Gorham 1995). Resistance is the ability of plants to adapt to salinity. It can be achieved by the ability of growing cells of a plant to avoid high ion concentrations or the ability of cells to cope with high ion concentration (Greenway and Munns 1980). Levitt (1980) characterized these mechanisms as avoidance and tolerance, and has used the term salt resistance to refer to a combination of tolerance and avoidance strategies. Examples of salt avoidance mechanisms include delayed germination or maturity until favourable conditions prevail; the exclusion of salt at the root zone or preferential root growth into non saline areas; compartmentalization of salt into secretion from specialize organelles such as salt glands and salt hairs or storage in older leaves (Hasegawa, Bressan, and Bohnert 2000). These tolerance mechanisms are discussed under separate headings.

2.4.1 Avoidance

This is shown by plants which escape from salt stress in their life cycle in a number of ways. They mostly coincide the active phase of their life cycle with favourable conditions. For example, they limit germination, growth and reproductive processes to specific seasons with high rainfall (Waisel 1972, Fitter and Hay 1987). For example some species germinate after heavy rainfall when salts are leached by the rain water, reducing the salinity to a minimum.

2.4.2 Tolerance

Tolerant plants have the ability to grow and complete their life cycle on saline substrates that contain a high concentration of salt. These plants maintain their metabolic activities even in the presence of high salt concentration. In saline conditions the tolerant plants have to meet two requirements i.e. osmotic adjustment and maintenance of the mineral elements needed for growth and functional metabolism (Jeschke 1984). They show

various physiological responses which enable them to complete their life cycle even in highly saline conditions. They have the ability to control ion uptake by roots and transport to shoots and leaves (Jeschke 1984, Chhipa and Lal 1993, Jacoby 1994, Shannon, Grieve, and Francoise 1994); to accumulate or exclude ions selectively (Greenway and Munns 1980, Ashraf and Fatima 1995, Cerda *et al*. 1995) to achieve selectivity in the xylem thus avoiding ions from xylem sap reaching the shoot from the root (Jeschke 1984, Lacan and Durand 1995) to compartmentalise ions at the cellular and at the whole plant level, e.g. accumulation in lower and older leaves, accumulation in roots rather than in the leaves (Leigh and Storey 1991, Jacoby 1994, Garcia-Agustin and Primo-Millo 1995) to utilise the accumulated ions to contribute to the cell osmotic potential (Poljakoff-Mayber, and Lerner 1994) and to accumulate so called compatible solutes such as proline, betaine, sorbitol and various carbohydrates, that reduce the osmotic potential of the plant and consequently allow continued water uptake (Greenway and Munns 1980, Kuiper 1984, Fedina, Tsonev. and Guleva 1993, Flowers, Troke, and Yeo 1997). In this model tolerance to salinity is effectively defined as the capacity to maintain an acceptable yield within a practical working range of salinity. As such practical salinity tolerance for crop production is a balance between yield and the physiological demand of dealing with salt stress. A plant that is capable of surviving extreme salinity has little value if the physiological demand of dealing with excess external and internal salt concentration reduces growth and yield below acceptable limits particularly if the salinity level tolerated exceeds the typical range to which it is likely to be exposed in most field situations. Plants can be found growing in saline environments equivalent to the salinity of seawater itself demonstrating that plant growth is not incompatible with salt.

2.4.3 Resistance

Resistant plants survive in high concentrations of salt without utilising salts in their metabolic functions. In this resistance mechanism, plants transport absorbed sodium to the shoot where the salt balance of the plants is maintained by the action of salt glands. These are specialised groups of cells on the leaf surface which actively transport sodium and chloride out of the cells onto the leaf surface, from which they are removed by the action of wind and rain (Sutcliffe 1962, Waisel 1972, Jacoby 1994). For example plants such as desert salt bushes (*Atriplex* species) have salt hairs which consist of a stalk and bladder cell. Salt is accumulated in the bladder cell which eventually bursts releasing the salt (Rajput and Sen 1991).

2.5 Mechanisms of salt stress resistance

High concentrations of sodium are toxic to most plant species, making soil salinity a major abiotic stress in plant productivity worldwide. Many crop species, which countless people rely for survival, are negatively affected. Physiological and biochemical research has shown that salt tolerance in halophytes depends on a range of adaptations embracing many aspects of a plants physiology, including; ion compartmentalisation, osmolyte production, germination responses, osmotic adaptation, succulence, selective transport and uptake of ions, enzyme responses, salt excretion and genetic control.

2.5.1 Selective accumulation or exclusion of ions

Both glycophytes and halophytes cannot tolerate large amounts of salt in the cytoplasm and therefore under saline conditions they either restrict the excess salts to the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions

(Zhu 2003). In general, exclusion mechanisms are effective at low to moderate levels of salinity whereas ion accumulation is the primary mechanism used by halophytes at high salt levels, presumably in conjunction with the capacity to compartment ion in the vacuole (Jeschke 1984). Glycophytes limit sodium uptake, or partition sodium in older tissues, such as leaves, that serve as storage compartments, which are eventually abscised (Cheeseman 1988). Apse *et al*. (1999) reported that removal of sodium from the cytoplasm or compartmentalization in the vacuoles is done by a salt inducible enzyme Na^+/H^+ antiporter. Inclusion of ions in the cytoplasm can lead to osmotic adjustment that is generally accepted as an important adaptation to salinity (Guerrier 1996). The decrease of leaf osmotic potential would compensate the salt induced lowering of water potential, helping to maintain turgor pressure and cell function under adverse water conditions. For example, under stress, sugar beet accumulated more inorganic ions in the leaves (Ghoulam and Fares 2001).

2.5.2 Synthesis of compatibles

The presence of salt in the growth medium often results in accumulation of low molecular mass compounds, termed compatible solutes, which do not interfere with the normal biochemical reaction (Hasegawa *et al*. 2000, Zhifang and Losecher 2003). These compatible solutes include mainly proline and glycine betaine (Ghoulam, Foursy, and Fares 2002, Khan, Ungar, and Showalter 2000). It has been reported that proline levels increase significantly in leaves of rice (Lutts, Kinet, and Bouharmont 1996) and in sugar beet (Ghoulam, Foursy, and Fares 2002). The increase in proline content in sugar beet was positively correlated with the level of salt tolerance. The proposed functions of proline under stress conditions include osmotic adjustment, protection of enzyme and membranes, as well as acting as a reservoir of energy and nitrogen for utilization during

exposure to salinity (Perez-Alfocea *et al*. 1993). Exposure to saline stress results in accumulation of nitrogen containing compounds such as amino acids, amides, proteins and polyamines, and their accumulation is frequently correlated with plant salt tolerance (Mansour 2000). According to Sakomoto, Murata, and Murata (1998), sub-cellular compartmentation of glycine betaine biosynthesis in rice is important for increased salt tolerance. These compounds have been reported to function in osmotic adjustment protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells and scavenging of free radicals. Other compatible solutes that accumulate in plants under salt tress include: (a) carbohydrates such as sugars (glucose, fructose, sucrose and fructans) and starch (Kerepesi and Galiba 2000, Parida, Das, and Mittra 2002). The major functions have been reported to be osmotic adjustment, carbon storage and radical scavenging (b) polyols are reported to make up a considerable percentage of compatible solutes and serve as scavengers of stress induced oxygen radicals and are also involved in osmotic adjustment and osmoprotection (Bohnert, Nelson, and Jensen 1995). According to Greenway and Munns (1980) salt sensitivity in non-halophytes may result from either (I) inability of osmoregulation, which may result from either an insufficient uptake of salt ions or a lake of synthesis of organic solutes being used as osmotic stress (ii) injury caused by inorganic ions which are absorbed by the cell and are not compartmentalized.

2.5.3 Control of ion uptake by roots and transport into leaves

Plants regulate ionic balance to maintain normal metabolism. For example, uptake and translocation of toxic ions such as Na^+ and Cl^- are restricted and uptake of metabolically required ions such as K^+ is maintained or increased. They do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the

driving force for transport (Zhu 2003). It is well documented that a greater degree of salt tolerance in plants is associated with a more efficient system for the selective uptake of K^+ over Na^+ (Noble and Rogers 1992, Ashraf and O'Leary 1996). It has been reported that a salt tolerant barley variety maintained a cytosolic Na^+ concentration 10 times lower than a more sensitive variety (Carden *et al*. 2003). Salt resistant tomato cultivars possess a high ability to select and translate the major nutrients (K^+, Ca^{++}, Mg^{++} and NO_3^-) to young leaves under moderate salinity (Perez-Alfocea *et al*. 1996). Nitrate selectivity over Cl^- in shoots has been correlated with salt tolerance in tomato cultivars (Perez-Alfocea *et al.* 1996). The use of plant ionic status to identify salt tolerance has been shown to be applicable (Ashraf and Khanum 1997) and its relationship with salt tolerance is considered strong enough to be explained as a selection tool in the breeding of salt tolerant cultivars (Omielon Epstein, and Dvovak 1991).

2.6 Managing salinity in agricultural production

Saline lands can be converted to more productive croplands by preventing the influx of salt water through proper management practices, correcting soil toxicities and nutrient deficiencies, and leaching the salt out the root zone. The reclamation costs can be reduced by growing salt tolerant cultivars. These practices are discussed below.

2.6.1 Management practices

Salinity can be restricted by a change in management practices. Munns *et al.* (2002) proposed that irrigated agriculture could be sustained by better irrigation practices such as adoption of partial root zone drying methodology, and drip or micro jet irrigation to optimize use of water. They suggested that salinity could also be contained by reducing the amount of water passing beyond the roots by reintroducing deep rooted perennial

plants that continue to grow and use water during the seasons that do not support annual crop plants. This may restore the balance between rainfall and water use thus preventing rising water tables and the movement of salt to the soil surface. Deep rooted perennial lucerne (*Medicago sativa*) has been found to lower the water table sufficiently to allow subsequent cropping (Ridley *et al*. 2001). Such practices will rely on plants that have a high degree of salt tolerance. Salt tolerance in crops will also allow the more effective use of poor quality irrigation water. Niknam and McComb (2000) suggested that trees could be planted to take up some of the excess salt since they have high water use and can lower the water table to reduce salt discharge into streams and prevent secondary salinization of the surrounding areas. However, it has not been proven to what extent tree planting would assist in preventing salt stress in neighbouring fields.

2.6.2 Organic amendments

Organic amendment is crucial for a fertile soil in a cropping system. Organic amendments of continuous cropping systems include animal composts, organic fertilizer and straw. Organic fertilizers refer to fertilizer derived from plant and animal residues. In other words, organic fertilizers are slow release fertilizers. In addition to supplying macronutrients, these fertilizers improve soil structure and water retention and contribute valuable trace elements. Because nutrients in organic fertilizer are released slowly over a long period of time, they are less likely to be leached than those from synthetic fertilizers. Most organic fertilizers must be decomposed by soil micro-organisms before they can release their nutrients to plants. Organic amendments may improve soil properties for application (Ginting *et al*. 2003), as only a fraction of the organic material is initially degraded and made available to plants and soil micro-organisms (Hades, Kautsky, and Portney 1996).

2.6.3 Zander

Zander is a fibre amended mineral material derived from naturally occurring organic deposits laid down over several thousand years. It develops by anaerobic decomposition associated with fresh water systems in many parts of the world. It is therefore ecologically sound and relatively small amounts can have a lasting effect in areas of the world affected by overgrazing, over-cultivation and desert encroachment (Zander Corporation 2007).

Zander has been successfully tested and recognized as being a highly effective material in supplying certain plant nutrients. In addition Zander possesses a high ion exchange capacity and can lock up soluble salts of elements so that they are not released either into the local environment or into the local vegetation. Zander can hold an amount of approximately twenty times the water content of free sand and release this water slowly. This helps plants rooted in Zander to survive for a very much longer time than those rooted in conventional composts without additional irrigation. Trials have shown that plants rooted in Zander-based media produce healthy growth over an extended period compared with plants rooted in non-Zander media (Zander Corporation 2007). The material of Zander is neither hazardous nor deleterious to the local environment, it is an organic rich material, and is clean, easy to handle and does not contain any substances deemed hazardous to health. It accumulates in lakes and bogs as a result of the build-up of silts mixed with decomposing vegetation. The tropic state (stage of development) can be observed; the layers, or strata being made up of different percentage of sandy, clay or carbonate minerals with organic components of decaying vegetation and aquatic fauna. The proportions will vary depending on the particular conditions that exist during different periods in the life of the pond. The natural moisture varies from 100 %

compared with the dry substance. It depends on the organic content, aggregate structure and compactness of the sediment. When Zander dries out it becomes porous with high specific surface area. The lower the initial ash level the higher the porosity of the powdered substance will be. It will also have a low specific weight. Zander contains important plant growth nutrients within its complex and a range of soluble organic acids that promote growth and sustain established plants (Zander Corporation 2007). According to Zander Corporation, typical analysis of Zander is shown in Table 2.3.

Table 2.3 Nutrient content in Zander (Zander Corporation 2007)

Element	Dry matter (%)
Organic matter	66.81
Inorganic component	33.19
Calcium	18.26
Silicon	9.41
Iron	2.68
Sulphur	1.39
Potassium	1.02
Phosphorus	0.15
Manganese	0.08
Sodium	0.01-0.1

2.6.4 Amelioration of salinity through fertilization

Salinity causes nutrient imbalance, resulting in lower concentration of the macro elements (N, P, K and Ca) in plant tissues. Hence, the most direct way to recover the normal nutrient concentration within the plant would be by raising their concentrations

in the root zone by higher fertilizer dosages. Many studies have shown that salt stress can be alleviated by an increased supply of calcium to the growth medium (Reusch *et al*. 1996, Ebert *et al*. 2002, Kaya *et al*. 2002). Depending on the concentration ratio, sodium and calcium can replace each other from the plasma membrane, and calcium might reduce salt toxicity (Reusch *et al*. 1996). Song and Fujiyama (1996) found that tomato plants grown in saline medium with supplemental Ca^{++} accumulated 40% less Na^{+} and more K^{+} than salinized plants without such supplement. Increased Na^{+} in the growth medium generally decreases the K^{+} content suggesting an antagonism between Na^{+} and K^{+} (Adams and Ho 1995). Addition of K^{+} to the nutrient solution has been found to raise K^{+} concentrations in the leaves and ameliorate the salinity stress effect (Lopez and Satti 1996). The effect of salinity on P in plants depends on P concentration in the nutrient solution. At high P concentration leaf injury has been interpreted as P toxicity induced by salinity (Awad, Edwards, and Campbell 1990). However, at low P concentration in the root medium, salinity was reported to inhibit P uptake by roots and translocation to the shoot (Martinez, 1996). At low P concentration in the root medium, supplementary P applied to the saline growth medium enhanced the capacity of tomato plants to regulate Na^{+} , Cl and K^{+} distribution, and improved plant growth (Awad, Edwards, and Campbell, 1990, Kaya *et.al*.,2001). Under salt conditions, the uptake of N by plants is generally affected and application of supplementary N has been found to ameliorate the deleterious effects of salinity (Gomez *et al*. 1996). The approach of raising fertilizer dosages may work on irrigation with water at low salt concentration. However, when water of high salinity is applied, the concentration of antagonistic ions required is so high that it causes a marked increase in the osmotic pressure of the soil solution, compounding the stress imposed by the salinity ions (Feogin 1985). Furthermore,

Grattan and Mass (1985) reported that in some species a very high concentration of nutrients could interact negatively with salinity ions, resulting in severe toxic effects.

2.6.5 Crop management

Some areas have naturally occurring salinity and salt tolerant crop plants may provide a better or perhaps the only way of utilizing these areas for food production. Salinity can possibly also be managed through biologically manipulating plants (Shannon *et al.* 1994). In addition to the land and water management practices for combating the salinity problem, an important factor will be to increase the salt tolerance of the existing crop species. One way to increase the salt tolerance is by increasing the threshold salinity levels of different crops. The threshold level is that salt level above which the productivity of a crop declines. This approach will also ensure the use of low to marginal quality water.

2.7 The genus *Acacia*

The genus *Acacia* in the family Leguminoseae sub-family Mimosodeae, tribe Mimosa is a large one encompassing more than 1200 species, of which more than 900 species occur only in Australia (Gates and Brown 1988, Fagg and Stewart 1994, Thomson, Turnbull, and Maslin 1994). The genus is widely dispersed in tropical and sub-tropical regions and is particularly well represented in Australia, South America, Asia and Africa. It is one of the most promising genera for arid, saline areas. Phyllodineous *Acacia* species have been introduced to the extremely drought prone Indian arid zone with very high temperature and wind velocities (Fox 1987). *Acacia saligna*, common names golden wreath wattle, orange wattle and blue-leaf, was previously known as *Acacia cyanophylla* and often still appears in the literature under this synonym. *A. saligna* is a native of Western Australia and has been introduced into many other

countries (Gutteridge and Shelta 1994), including Uruguay, Mexico, Libya, Iran, Iraq, Jordan, Syria, Greece and Cyprus (NAS 1979). *A. saligna* has been planted in some places for firewood although some people consider it to have a light, sappy wood (NAS 1980). In Queensland, in trials, *A. saligna* reached more than 6 m in height in less than 4 years, although with a high mortality and a high proportion of woody inedible stem material (Gutteridge and Shelta 1994). Where it is planted for fuel in Mediterranean countries, annual yield can vary from 1.5-10 m^3 ha^{-1} and the fuel wood is harvested on a five year rotation (NAS 1980). In Tunisia the wood has successfully been processed into particle board (El-Lakany 1987). This species is useful for windbreaks and especially for sand dune fixation which is one of the main reasons for its widespread introduction to other countries. The phyllodes of *A. saligna* are reported to be palatable and non toxic to livestock (Crompton 1992) and have been recommended as a supplementary feed for sheep and goats, although they are not suitable for ruminants. The phyllodes have a moderately low digestibility but high levels of crude protein (Vercoe 1989). According to Hall and Turnbull (1977), *A. saligna* was also planted in the past for tannin production. When damaged the bark exudes large amounts of acidic gum. Crompton (1992) states that this gum has promise for use in pickles and other acidic food stuffs. In Australia *A. saligna* has been used in the rehabilitation of sand mining areas (Hall and Turnbull 1977). Plantation legume trees and shrubs have attracted considerable interest as multipurpose species in a wide range of climatic zones (Harris 1988, Harris *et al.* 1989). Although *A. saligna* thrives on a wide range of soil types, it is outstanding on sandy coastal plains and sand dunes, making it a plant extensively used in sand dune stabilisation (Gutteridge and Shelta 1994) and mining site rehabilitation (Simmons 1988). It grows well in areas with annual rainfall as low as 250 to as high as 1200 mm, but prefers a range of 350-600 mm. It is moderately tolerant of soil salinity (Gutteridge

and Shelta 1994) as well as being tolerant of salt laden wind (Simmons 1988). *A. saligna* grows best where the winter and summer temperature means are between 13 and 30^{o}C. It grows at altitudes ranging from near sea level to 300 m. This tree can tolerate all desert environmental conditions and gives successful growth under saline soils conditions and when irrigated with saline water (Sheha 1984). *A. saligna* is easy to propagate, it is fast growing with an abundance of leafy foliage and it recovers quickly after annual lopping. It is often cultivated in saline soils and mostly irrigated with saline underground water that would otherwise be valueless. It may survive and grow on sites receiving as little as 20 mm of rain annually (El Lakany 1987).

2.7.1 Morphology of *Acacia saligna*

A. saligna is a dense bushy shrub (Gutteridge and Shelta 1994) or small tree that grows 2.5- 8 m tall (Simmons 1988). It has long straggling branches (Gutteridge and Shelta 1994); which are grey to reddish brown in colour. The foliage varies in colour, either green or blue green (Simmons 1988) with long phyllodes (up to 20 cm) (Gutteridge and Shelta 1994) that are either curved or straight (Simmons 1988). During spring it is usually characterised by drooping branches that contain an abundant number of yellow flowers (NAS 1979).

2.7.2 Germination

In a strict botanical sense germination has been defined as the process which starts with inhibition, proceeds through intermediate phases of enzyme activation and mitosis, and ends with elongation of the radical and seedling emergence including both the pre germination and the post germination growth of the seed and seedling before emergence at the soil surface (Sylvester-Brdadley And Roebuck. 1985). The definition from the

international rules for seed testing (ISTA 1966) states that germination is the emergence of those essential structures (root and shoot), which, for the kind of seed being tested, indicate the ability to develop into a normal plant under favourable conditions in soil. Some authors simply define germination as the first emergence of the radical (Collies, George, and Sands 1959) or the emergence of root and shoot (Currie 1984) from the seed. For Mayer and Poljakoff-Mayber (1989) germination of the seed of higher plants may be regarded as that consecutive number of steps which causes a quiescent seed with a low water content to show a rise in its general metabolic activity, and to initiate the formation of a seedling from the embryo. The exact stage at which germination ends and growth begins is extremely difficult to define. This is particularly difficult because germination is identified by the protrusion of some part of the embryo from the seed coat, which is itself already an indication of growth. There is no general rule as to which part of the embryo first pierces the testa in many seeds this is the radical and therefore germination is frequently equated with root protrusion. However, in some seeds it is the shoot with protrudes first.

2.7.3 Seedling production

A. saligna seeds can be collected by hand from selected trees of good palatability and production. Seed germination is the driving force determining the existence and establishment of plants. The presence of excess salts is one of the critical factors adversely affecting seed germination under many natural conditions. Seeds are sown in the upper surface of the soil for their germination, where the salt concentration may be higher than in other parts of the soil profile. According to Pasternak, Twersky, and Malachi (1979), it would be advantageous for plants to be able to germinate at high salt concentrations and have a long and fast growing root system. In this way they would

extend their roots rapidly to non-saline or less saline soil environments and be more likely to emerge and grow better during the later stages of growth. However, many plants have seeds which when germinated at high salt concentrations, have a longer germination time or slower growth of the seedling root system and thus have a high rate of mortality. Shalhevet, Huck, and Schroeder (1995), working with maize and soybeans suggested that once the main root is established there is a minimum effect of salinity on root growth.

2.7.4 Seed coat permeability

Seeds of *Acacia* species are known to have hard coats that completely prevent the imbibition of water and exchange of gases, thus preventing initiation of the germination process (Khasa 1993). Such physical seed-coat dormancy occurs most frequently in species adapted to alternating dry and wet seasons. The seed coat is a barrier that must be broken to enable the embryo to obtain water needed to reactivate its metabolism and start growing. Before imbibition and germination can occur, the seed coat must be rendered permeable. In general, by virtue of their hard seed coat, which minimises moisture exchange and the loss of stored reserves through respiration, *Acacia* seeds retain their viability well for many years and present few storage problems. Seeds of some species can remain viable after 50 years in the field (Moffect 1952). The possession of a hard seed coat has some ecological advantages; it favours the accumulation of a persistent seed bank in the soil by preventing germination of viable seed in the soil for long periods. This spreads germination over time, increasing the chance that some seeds will germinate, establish and complete the life cycle successfully (Bewley and Black 1994). In unpredictable environments, such as those of arid Australia, germination is often a high-risk event; possession of a hard seed coat thus

reduces this risk by avoiding depletion of the seed bank following a single large rainfall event. The hard seed coat is waxy and water repellent. It can also be considered as having evolved to withstand unfavourable conditions such as heat caused by fire, strong teeth of dispersing animals or passage through their gut, severe drought and mechanical damage. Germination impedance might sometimes be the result, not of inadequate moisture but of seed coat impermeability. Impermeability of seed coats is common in many seeds, especially among the legumes. Water is needed by the seed and by the seedling at all stages. Uptake of water by the seed (imbibition) is one prerequisite for breaking dormancy. The extent to which imbibition occurs is determined by two factors; the composition of the seed and the permeability of the seed coat or fruit in the environment (Mayer and Poljakoff-Mayber 1989). The larger the seed the more water it will imbibe, the greater therefore, the zone of water depletion in the soil around the seed and the further the mean distance that the water must travel (Currie 1984). This zone of depletion is greater in a dry soil than in a wet soil (Dasberg and Mendel 1971).

To accelerate germination of *Acacia* seeds, various pre-treatment methods have been assayed including soaking in boiling water, and sulphuric acid scarification (Doran, and Gunn 1987). One of the simplest and most direct methods is to cut, drill or file a small hole in the seed coat before sowing. This was done on *Acacia* seeds in Honduras (Willian 1985). In laboratory trials in Sweden, sandpaper scarification followed by a 3-h cold water soak was the most effective treatment for *Acacia farnesiana.* Hence, it is difficult to prescribe an optimum treatment that is highly effective in stimulating germination in most *Acacia* seeds (Doran and Gunn 1987). However, it is vital to practise efficient, easily applied seed pre-treatment methods that can be used if large numbers of plants are required. The imbibition pressure developed by seeds may reach

hundreds of atmospheres, and in colloids such as agar or gelatine, pressures of many hundreds of atmospheres have been measured (Mayer and Poljakoff-Mayber 1989). The imbibition pressure is of great importance in the process of germination as it may lead to the breaking of the seed coat and also to some extent makes room in the soil for the developing seedling. The magnitude of the imbibition pressure is also an indication of the water retaining power of the seed and therefore determines the amount of water available for dehydrating the seed tissues during germination. Seeds of *A. saligna* are normally treated with boiling water, but nicking the seed coat, soaking in sulphuric acid (H_2SO_4), and exposing the seeds to dry heat are also effective (Khasa 1993). For small to medium-sized seeds or large quantities of seeds, the hot water treatment is more useful than scarification. For this treatment, seeds should be soaked/dropped into about six times their volume of 80-90 ºC pre-heated water (rain water is desirable if it is near neutral in pH). The seeds should than be left to cool and soak in the water for 12 to 24 h, after which they are ready for sowing. The container used for this treatment should not be made of aluminium as it may be toxic to the seeds. Also, softened water should not be used since the amount and ratio of salts may be toxic to the seeds. Another and more drastic hot water treatment is used especially for thick or hard-coated seeds. For this treatment, the seeds should be placed in vigorously boiling water for a specific length of time depending on the species, then immediately removed from the boiling water and cooled in cold water. Larsen (1964) conducted experiments on seed pre-treatment of *A. saligna* where seeds were treated by (1) placing in boiling water and cooling to room temperatures (23^{o}C); (2) boiling in water for 5 or 10 min and cooling to room temperature; or (3) soaking in concentrated H_2SO_4 at room temperature or 50°C for 30, 60, 90 or 120 min followed by washing in running water for at least 10 min. Seeds from all treatments and untreated controls were germinated on filter paper in the dark at 5, 10,

15, 20, 25, 30 and 35 ºC. All pre-sowing treatments increased percentage germination and rate of germination. The most effective treatments were H_2SO_4 at 50°C for 60 or 90 min followed by germination at 15 or 20°C.

2.7.5 Establishment

Establishment is defined as the period between sowing the seed and the emergence of autotrophic seedlings capable of continued, normal growth, and it is the outcome of an interaction between the seeds and the environment into which they are sown (Perry 1984). It has two components: (1) seed germination, and (2) seedling growth through the covering soil (or emergence). Establishment of crops under field conditions can be very unpredictable both in the time of seedling emergence and in the percentage of seeds which produce established seedlings. Pre-emergence, losses which can be as much as 25-50% (Salter 1984), can be caused by any number of uncontrollable environmental factors which affect seed germination and establishment under field conditions. In addition, recently emerged seedlings can be vulnerable to adverse weather and soil conditions and to attacks of pests or pathogens which can further reduce the plant population. As the total yields of many crops are closely related to plant density, failure to achieve the desired population will result in lower yield (Salter 1984). Treated seed should be planted to a depth of 0.5 cm. Seedlings can be produced either by direct seeding or in a nursery. A nursery phase of 10-12 weeks is recommended. Soil should not be allowed to dry between sowing and germination. Young plants require protection from grazing animals and other hazards such as drought (NAS 1980). The successful establishment of young seedlings depends on the physiological and biochemical factors of the seed. There is a strong interaction between various environmental factors and the germination of seeds. Intolerance of seeds to these factors may result in physiological

and biochemical disorders leading to abnormal seed germination. The presence of saline conditions in the germinating environment may be a critical factor in seed germination and can prevent the plant species from successfully colonizing an area. Seeds fail to germinate in saline soils because the soil micro-environment often has a high salt concentration due to the upward movement of water and a high rate of evapotranspiration (Pasternak, Twersky, and Malachi. 1979). It is a well known phenomenon that salinity can reduce the germination percentage and delay germination in many plant species. In general, osmotic and /or toxic effects are responsible for salt injury.

2.7.6 Cultivation

A. saligna can be grown in plastic bags and transplanted in the cool season as soon as rainfall permits; in arid areas some watering may be necessary to get the plants established. Spacing varies greatly according to the system of management foreseen. Nefzaoui (1997) mentions very widely spaced rows intercropped with barley in dry parts of Tunisia and discusses its use along with *Atriplex* and fodder cactus in feeding systems.

2.7.7 Emergence

Successful emergence of the plumule is perhaps more important than root growth at the early stage because until photosynthesis starts, the seedling must rely entirely on energy stored within the seed (Currie 1984). Percentage emergence is similar to and closely correlated with percentage germination in favourable soil conditions, but when conditions are less favourable, emergence is reduced and correlations with germination become poorer (Harris, Hamdi, and Terry 1987). Establishment of crops under field conditions can be very unpredictable both in the time of seedling emergence and in the

percentage of seeds which produce established seedlings. At the farmer level, germination as scientifically defined becomes an obscure concept and emergence, which is the first visible and quantifiable result of sowing, takes over; this explains why emergence is usually mistaken for germination.

2.8 Seed factors

The main seed factors that influence establishment are viability, purity, health and vigour, and they can be tested before the seeds are sown (Perry 1984). The size, shape, structure and composition of seeds can determine their germination behaviour in different environments. The seed of angiosperms (higher plants) consists of an embryo and testa (or seed coat) (Mayer and Poljakoff-Mayber 1989). The embryo consists of a radicle, a plumule or epicotyl, one or more cotyledons and a hypocotyl which connects the radicle and the plumule. The process of germination leads eventually to the development of the embryo into a seedling. A seedling is classified as epigeal in which the cotyledons are above ground and are usually photosynthetic and hypogeal in which the cotyledons remain below ground. The testa is a structure of considerable importance because it forms the barrier between the embryo and its immediate environment, and one of the most interesting features of the testa is its impermeablility to water.

2.8.1 Seedling environment

The seed or seedling prior to emergence may be subject to a variety of environmental stresses. Khan *et al.* (1979) listed 19 physical, mechanical, chemical and biotic sets of factors that can affect seed performance. The list includes; supra- and sub-optimal temperature; unfavourable light conditions; drought; flooding; unfavourable gaseous environment; soil texture and composition; depth of soil; crusting of soil surface;

composition of soil and inadequate seed soil contact; colloidal content; salinity, pesticides, herbicides and their residues; toxic gases; soil pH; fertilizers; insects; fungi and bacteria and weeds. The first requirement of any seed bed is that is should provide the right conditions to break the dormancy of the seed. Thereafter, germination, emergence and early growth of the seedling will bring new requirements. The requirements of the seed are few and mostly simple. Seeds need adequate amounts of water and air, a suitable temperature, a facility for the root and shoot to move freely when the seed germinates and available nutrients (Currie 1984).

2.8.1.1 Effect of light

Light is abundant usually only on the surface of the soil. In sandy soils, light penetrates a short distance into the soil, although its intensity falls rapidly. In heavy soils, light will not penetrate at all. In cases where the soil is covered by water, light will penetrate a considerable distance provided the water is clear (Mayer and Poljakoff-Mayber 1989).

2.8.1.2 Effect of sowing depth

Sowing depth is the mean vertical distance (cm) of seeds below the soil surface, after the seedbed has settled (Sylvester-Bradley and Roebuck 1985). Soil depth will influence aeration as well as the penetration of water and light. Sowing depth may affect yield through its effect on date of emergence and percent of plant emerging. Sowing deeper than necessary exposes the seeds to possible anaerobic conditions during wet periods and inevitably extends the pre-emergence growth period (Perry 1984). Heydecker and Coolbear (1977) suggested that although increased depth of sowing might increase root anchorage and moisture availability, these advantages could be offset by inferior aeration, and increased mechanical obstruction and the possibility of increased microbial

attack on longer hypocotyls. Erickson (1946) contends that both germination and vigour of seedlings are directly associated with seed size, as seedling vigour was decreased by increasing the planting depth of small alfalfa seeds, and, conversely, a higher percentage of vigorous seedlings resulted when large seeds were planted deep. Bolton (1983) and Johnson (1983) suggested that the large seeded cereal crops should be seeded at a maximum depth of 5 to 6 cm (Anderson 1974) and small seeded crops should be seeded at 2.5 cm or less.

2.8.1.3 Effect of soil temperature

Different seeds have different temperature ranges within which they germinate. At very low and at very high temperature, the germination of all seeds is prevented. In the range of temperature within which a certain seed germinates, there is usually an optimal temperature below and above which germination is delayed but not prevented. Hegarty (1973) stressed that the rates of seed germination and seedling emergence are greatly affected by temperature. According to Mayer and Poljakoff-Mayber (1989), imbibition proceeds more rapidly at higher temperatures. In some species, however, high temperatures can induce a specific dormancy response which protects the seed against damage but which prevents further germination until dormancy has been lost or broken.

2.8.1.4 Influence of soil depth on soil temperature

Usually the upper layers of the soil show wide fluctuations in temperature and as greater depths are reached conditions become more and more constant through the year (Marshall and Holmes 1988). The temperature at depth, as the temperature at the surface, is sinusoidal. It varies with the same frequency; the lag behind the surface

temperature increases with depth and amplitude decreases with depth (Currie 1984). Generally, the surface 5 cm of soil in the sub-arid zone has a maximum temperature of 40 to 50 °C (Kanemasu *et al*. 1990). Willcocks (1982) even recorded a soil surface temperature of 55°C under grass, and a high of 70°C in bare soil in Botswana. While the amplitude of the daily temperature variation is greater at the surface of a loose dry soil than of wet soil, the variation at depth is greater in a wet soil than in a dry one (Currie 1984).

2.9 Planting *Acacia saligna*

Livestock play an important role in most small scale farming systems throughout the Libyan environment. They provide traction to plough fields, manure which maintains crop productivity, and nutritious food products for human consumption. In most small-scale farming systems livestock graze in pastures or woodlands feeding on grass and other herbaceous plants. During the winter season these lands provide adequate forage to maintain productive animals. In the dry summer, however, the quantity and quality of forage greatly decreases and is generally low in nutritional value. To avoid these problems farmers must provide their animals with quality feeds to augment dry season forages. One option is to supply expensive concentrates or supplemental feeding. For most small scale farmers this is not possible due to high costs and limited availability of supplements. A more practical option is for farmers to establish fodder trees such as *Acacia* species. *Acacia* produce high quality fodder which grows in low rainfall in dry regions. Plants can be utilized all year, but are designed to bridge the forage scarcity of annual dry seasons. *A. saligna* plants are legumes, usually trees or shrubs. The relatively deep roots of these woody perennials allow them to reach soil nutrients and moisture not available to grasses and herbaceous plants.

Chapter 3

Influence of treatments to break seed Dormancy and Effec of seed size and sowing depth on germination, seedling growth of *Acacia saligna*

3.1 Introduction

Seeds of many tree species germinate when they are subjected to favourable conditions of moisture and temperature, while many other species possess some degree of seed dormancy. Pretreatments to overcome physical seed coat dormancy are designed to split the seed coat on order render it permeable without damaging the embryo. This is due to their water impermeable testa, which exerts a physical exogenous dormancy (Holmes, McDonald, and Juritz 1987). Seeds of *Acacia* species are known to have hard coats that completely prevent the imbibition of water and exchange of gases, thus preventing initiation of the germination process (Khasa 1993). *Acacia* seeds, therefore, will not germinate easily when placed under favourable conditions for germination. Such physical seed coat dormancy occurs most frequently in species adapted to alternating dry and wet seasons. The seeds usually have a fleshy outgrowth called an aril where the seed attaches to the pod. The arils may be white or brightly coloured and are often attractive to ants or birds that help disperse the seed (Entwistle *et al.* 1996).

To accelerate germination of *Acacia saligna* seeds, various pre-treatment methods have been assessed including soaking in boiling water or concentrated acids and scarification (Doran and Gunn 1987). The proportion of hard-coated seeds in a sample may be influenced by environmental conditions during the growth of the plant, the degree of the maturation of the seeds when collected, and duration and type of seed storage (Willian 1985). Very few studies have been done on overcoming the germination problems of *Acacia* species that being utilised in Libya.

Seed size may be an important factor in seedling survival as it is likely to be affected by the quantity of metabolic reserves in the seed, seed distribution; seed water relations; persistence in the soil bank; seedling establishment; and plant fitness (Bonfil 1998). On the other hand; small seeds are characteristic of species that have persistent, dormant seed banks in the soil. Small seeds may facilitate burial and protection from seed pests, assist in dispersal and enhance germination rate. Small seeds tend to have a higher surface-volume ratio than larger ones (Wulff 1986). One of the most effective adaptations for ensuring successful seedling establishment is the presence of large seeds. Seedlings from large seeds contain greater metabolic reserves for the embryo and thus can attain a larger initial seedling size (Westoby, Jurado, and Leisman 1996). The larger reserves in heavier seeds may allow more pre-photosynthetic growth of the seedling supporting large embryos with substantial food reserves, which enable a seedling to achieve better growth of both root and shoot. Enhanced nutrient reserves in large seeds and their translocation from cotyledons to the seedling body during early growth can reduce the reliance of the seedling on external supplies of nutrients, a distinct advantage in infertile soil. Vaughton and Ramsey (2001) found that seed size determines the initial area of cotyledons and later determines the rate of early vegetative growth.

Sowing depth may affect yield through its effect on date of emergence and percentage of plants emerging. Sowing deeper than necessary exposes the seeds to possible anaerobic conditions during wet periods and inevitably extends the pre-emergence growth period. Perry (1984) suggested, however, that increased depth of sowing might increase root anchorage and moisture availability. The current work aimed to study the effect of several methods of physical dormancy breaking on final germination and rate of germination to establish efficient methods for removing hard seed coat dormancy of *A. saligna*. One aim was to develop a better understanding of the effect of seed size on the

effect of seed size as well as the depth of seed sowing on the germination efficiency of *A. saligna* to determine the most condition germination condition and seedling growth potential.

3.2 Materials and methods

Seeds of *Acacia saligna* (Labill.) H.L. Wend. Were supplied from Setropa BV, Troelstralaen 4, 1272 JZ Huizen, The Netherlands in 1 kg quantities in air-tight aluminium foil bags. This experiment was carried out from November to December 2007 in a laboratory at Coventry University, England. Seeds were measured with Vernier calipers and graded into three groups; large (length ≥ 8 mm, width ≥ 3 mm), medium (length < 8 mm and ≥ 5 mm, width ≥ 2.5 mm), and small (length < 5 mm and ≥ 3 mm, width ≥ 2 mm).

3.2.1 Mechanical and chemical scarification

Seeds were divided into two groups, with arils and without arils. Germination of unscarified seeds with and without arils was tested without the application of dormancy breaking methods to assess the effects of arils. In addition seeds with arils were scarified by mechanical and chemical means to break their hard seed coat dormancy. Mechanical scarification was achieved by removing a small section of the seed coat at either the aril end of the seed or the opposite end. Sand paper scarification was carried out using coarse sand paper (aluminium oxide (45-PG)). Seeds were abraded by rubbing the side opposite the embryo between two sheets of sand paper for 10, 20, 30, 40 or 50 min. Chemical scarification involved the immersion of seeds in an excess of 98% sulphuric acid for 20, 40, 60, 80 or 100 min. Seeds were immersed in approximately 100 ml of sulphuric acid per 100 seeds, in 250 ml beakers at room temperature. The seeds were stirred occasionally to prevent them sticking together and to ensure contact with the

acid. After scarification, the seeds were removed from the acid by pouring through a plastic sieve and were then washed for 10-20 min under running tap water. When all traces of acid had been removed, the seeds were blotted dry and allowed to air dry at room temperature.

3.2.2 Boiling water scarification

Seeds were treated with boiling water to break their hard seed coat dormancy. Seeds were placing in boiling water and cooled to room temperature. The volume of boiling water was approximately 100 ml of water per 100 seeds, in 250 ml beakers at room temperature. The treatments applied were. Treatment 1: Control treated by adding 100 ml of tap water to the seeds, Treatment 2: Addition of 100 ml boiling water to seeds and seeds left to cool in water for 30 min, Treatment 3: As 2 but after 30 min the boiling water was replaced by another 100 ml of boiling water, Treatment 4: As 3 but after 60 min the boiling water was replaced by a further 100 ml of boiling water, Treatment 5: As 4 but after 90 min the boiling water was replaced by another 100 ml of boiling water.

3.2.3 Seed germination

Seeds were germinated in 9 cm plastic Petri dishes containing two Whatman No 1 filter papers and 10 ml of distilled water. Twenty seeds were sown per dish and five replicate dishes were used for all treatments. The design adopted was a completely randomized design. Seeds were incubated in the dark at a constant 15 ^{o}C. The seeds were observed daily and scored as germinated when approximately 2 mm of radicle had emerged through the testa. Germinated seeds were removed from the Petri dishes daily. Final germination was calculated as the maximum germination obtained when no further germination took place for several days. Germination rate was recorded as $1/t_{50}$ where t_{50} is the number of days required to reach 50% of the final germination.

3.2.4 Dormancy breaking test

A germination test was carried out to investigate the effect of seed size and boiling water treatment on germination. Seeds were treated by pouring 200 ml of boiling water onto 400 seeds and leaving them to cool. Boiling water was applied one, two, three and four times at 30 min intervals (boiling water used because it is considered as the most practical treatment to breaking dormancy of *A. saligna* seeds). Seeds treated once with cold water for 30 min were used as a control. For the greenhouse experiment seeds of each seed size x dormancy breaking treatment were germinated as described above, but after 24 h the seeds were checked to see if they had imbibed water, as indicated by swelling of the seed, and imbibed seeds were sown at 30 mm depth in sandy soil. The greenhouse experiment was carried out at Coventry University, England during February and March 2007. The temperature during the entire growth period was maintained at around 25-27^{o}C and the relative humidity ranged from 40 to 60%. Tall profile pots with a diameter of 150 mm were used. The bottom of the pots contained a piece of filter paper cut to size to prevent the soil running out when dry. Sandy soil was added to partly fill the pots, leaving sufficient room to bury the seeds at 30 mm depth. Twenty imbibed seeds were sown in each of four replicate pots for each seed size x dormancy breaking treatment. The pots were arranged on a bench in the greenhouse in a completely randomized design. Pots were watered as required to maintain a moist soil. Seedling establishment was assessed after 7 weeks as the number of plants which has emerged and survived until the end of the experiment.

3.2.5 Effect of sowing depth and seed size on seedling establishment and growth

For the second greenhouse experiment, dormancy of seeds of each of the size classes was broken by three applications of boiling water at 30 min intervals. Seeds were

germinated as described above and after 24 h the seeds were checked to see if they had imbibed water, as indicated by swelling of the seed. Thereafter, 80 imbibed seeds of each size class were selected for each of four sowing depth treatments, 10, 20, 30 or 40 mm depth. Twenty imbibed seeds were sown in each of four replicate pots for each of the four sowing depth treatments. Thus this part of the experiment comprised three seeds sizes, four sowing depths and four replicates of 20 seeds for each size x depth combination. The pots were arranged on a bench in the greenhouse in a completely randomized design. Seedling establishment was assessed after 7 weeks as the number of plants which had emerged and survived until the end of the experiment.

The shoot and root lengths of surviving seedlings were measured when harvested after 7 weeks. Shoot and root dry weight per plant was measured after drying in an oven at 80ºC for 48 h and cooling in a desiccator. Seeds that had not germinated and seedlings that had died were excluded from the dry weight estimates. This experiment was carried out twice, once with irrigation with distilled water and once with tap water. One of the advantages of watering plants with distilled water is that it does not add chlorine or fluorides that are often found in tap water.

3.2.4 Statistical analysis

The significance of differences between means was tested by one way ANOVA using the Minitab Computer Package 15 followed by the calculation of a least significant difference for all pairs of comparisons using Tukey's test at $p \leq 0.05$. Final germination data were arcsine transformed before analysis. Germination percentage data were arcsin transformed to increase homogeneity of variance prior to analysis.

3.3 Results

3.3.1 Germination % of break dormancy

3.3.1.1 Mechanical treatments

The analysis of variance revealed that both germination percentage and germination rate of *A. saligna* was significantly affected by the pre-germination treatment. Figure 3.1 shows that mechanical scarification by cutting the seed testa with nail clippers significantly increased seed germination compared with untreated seeds. There was no significant difference between seeds cut at the aril end or the opposite end.

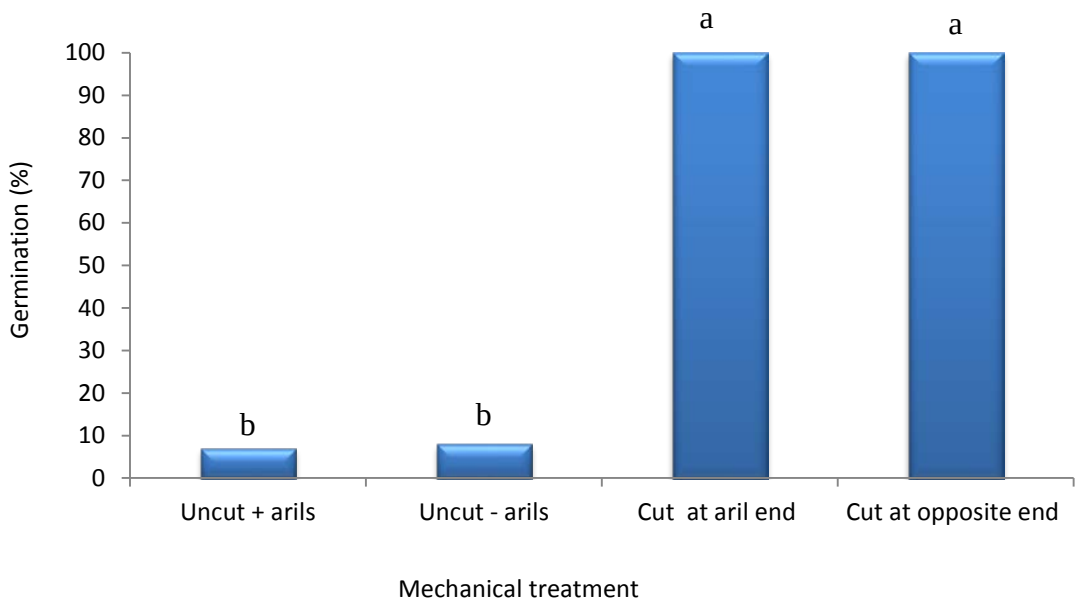

Figure 3.1: Effect of cutting on seed germination. Means without a letter in common differ significantly at $p \leq 0.05$ using Tukey's test on arcsine transformed data.

3.3.1.2 Soaking in boiling water

Figure 3.2 shows that treating *A. saligna* once with boiling water significantly improved seed germination compared with the control. Boiling water applied twice (60 min) significantly improved germination compared with once (30 min). Increasing the time of treatment (90 min) further significantly increased germination level. However, treatment

of seeds with boiling water four times (120 min) resulted in significantly less germination than those treated three times, but was still significantly higher than the other treatments.

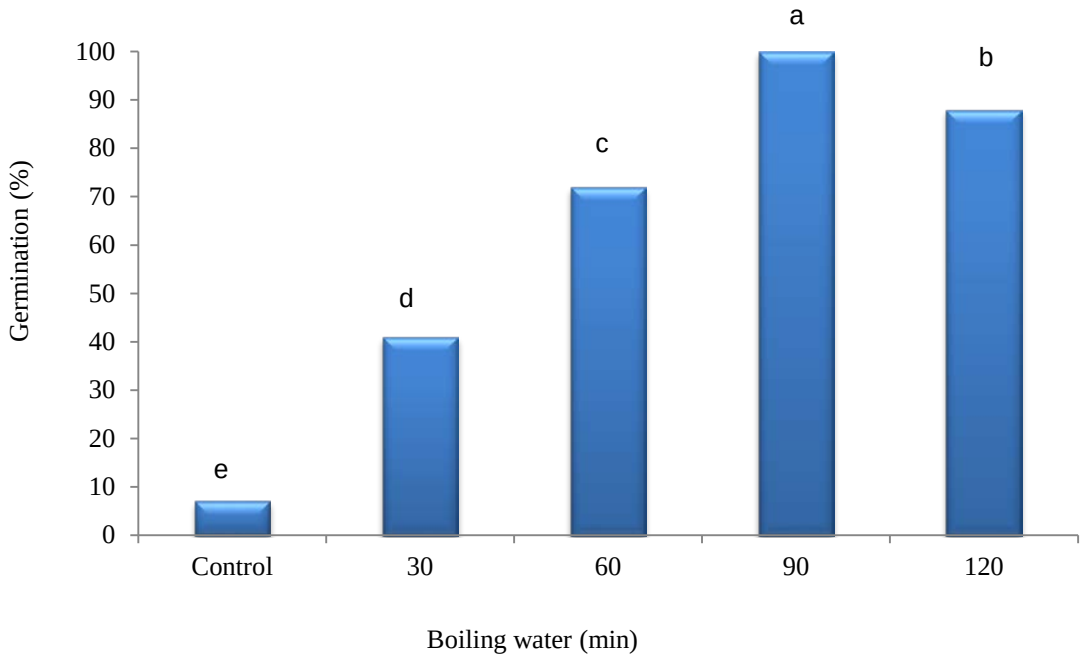

Figure 3.2: Effect of number of boiling water treatment on seed germination. Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test on arcsin transformed data.

3.3.1.3 Soaking in sulphuric acid

Figure 3.3 shows that soaking using sulphuric acid significantly increased germination level compared with the untreated control. However, there was significant difference between seeds soaking in acid for 20 min and those for 40 min in germination level. Germination was increased significantly with the time of treatment up to 60 and 80 min, but decreased again when treatment was increased to 90 min and no significant difference with 40 min and those for 90 min.

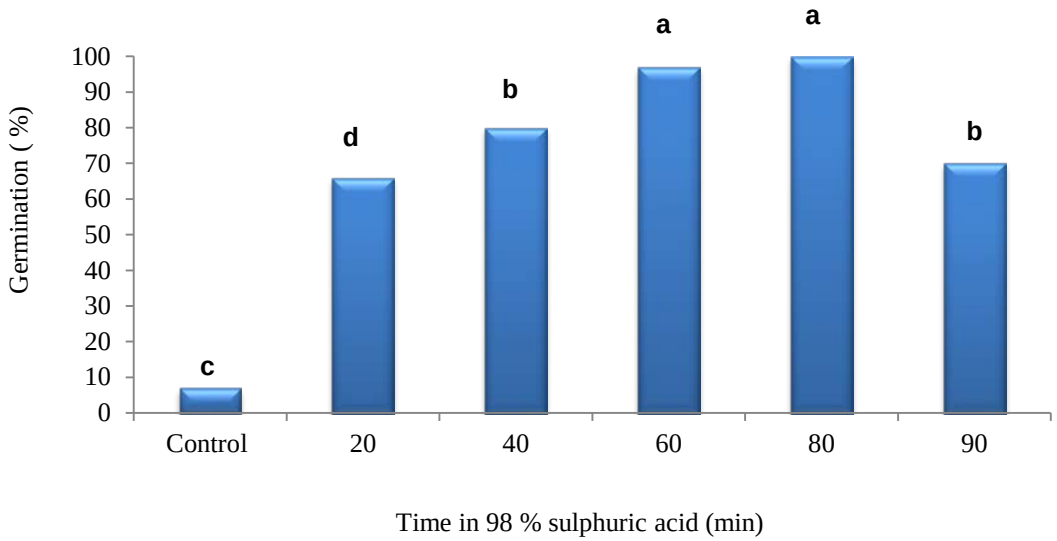

Figure 3.3: Effect of H_2SO_4 treatment on seed germination. Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test on arcsin transformed data.

3.3.1.4 Scarification of seeds

Figure 3.4 shows the effect of sand paper treatment on germination. Germination significantly increased with the time of scarification up to 30 min, but more than 30 min had no further significant impact on germination.

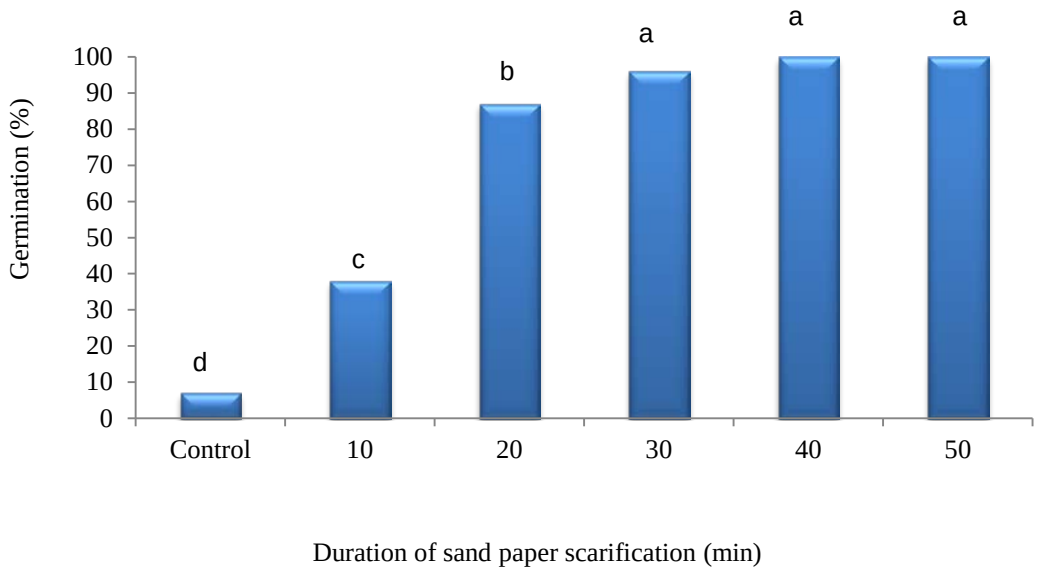

Figure 3.4: Effect of sand paper scarification on seed germination. Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test on arcsin transformed data.

3.3.2 Germination rate

3.3.2.1 Mechanical treatments

Figure 3.5 shows that mechanical scarification by cutting the seed testa with nail clippers led to significantly increased germination rate compared with that of untreated seeds. Furthermore, there was a significantly lower germination rate of seeds cut at the aril end than of seeds cut at the opposite end. There was no significant difference in the germination rate of uncut seeds with or without arils.

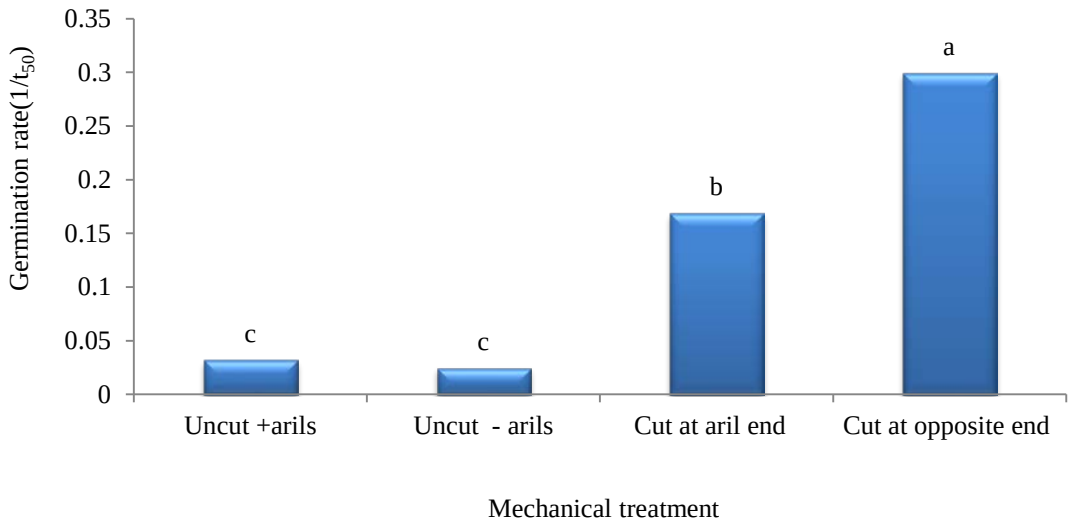

Figure 3.5 Effect of cutting on germination rate. Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test.

3.3.2.2 Soaking in boiling water

Figure 3.6 shows that treating seeds of *A. saligna* once (30 min) or twice (60 min) with boiling water did not significantly improve germination rate compared with the control. However, increasing the frequency of boiling water treatments to three times (90 min) significantly increased germination rate compared with the control and shorter treatments, but treatment of seeds with boiling water four times (120 min) was less effective than three times.

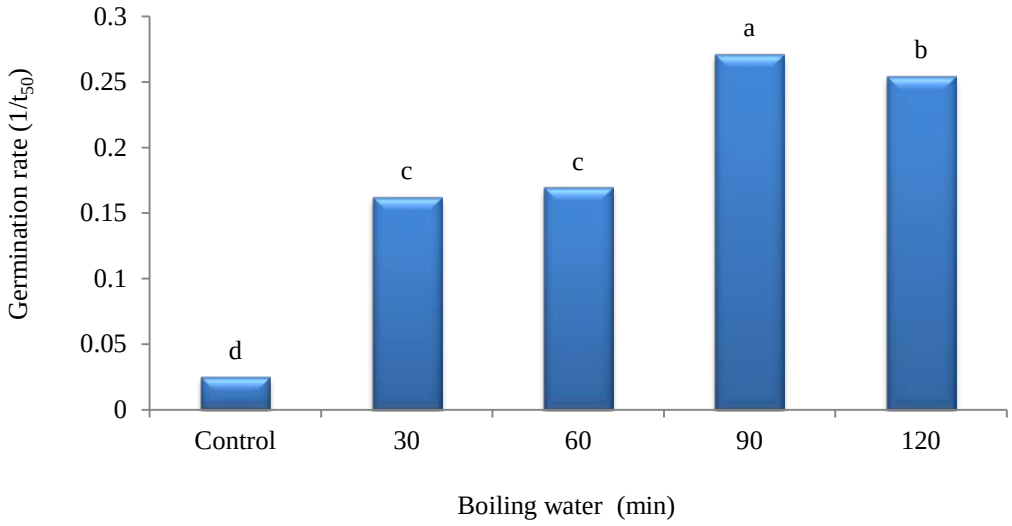

Figure 3.6: Effect of boiling water treatment on germination rate. Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test.

3.3.2.3 Soaking in sulphuric acid

Figure 3.7 shows that soaking in sulphuric acid for all times tested, significantly increased germination rate compared with the control. There was no difference in germination rate between seeds soaked for 20, 40 min, but germination rate decreased significantly above 60 compared with 20 and 40 min treatments.

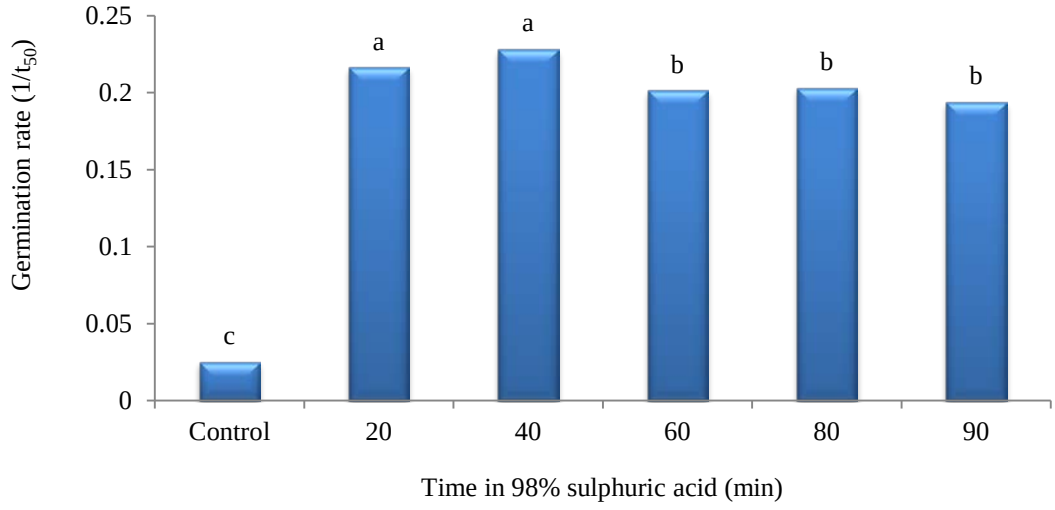

Figure 3.7: Effect of H_2SO_4 treatment on germination rate Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test.

3.3.2.4 Scarification of seeds

Figure 3.8 shows that sand paper scarification had a significant effect on germination rate, compared with unscarified seeds. However duration of scarification had more variable results. Scarification for 20 min did not significantly increase germination rate above seeds treated for 10 min. Scarification for 30 min significantly increased germination rate compared with 10 and 20 min germination rate decreased significantly with 40 and 50 compared with 30 min treatment.

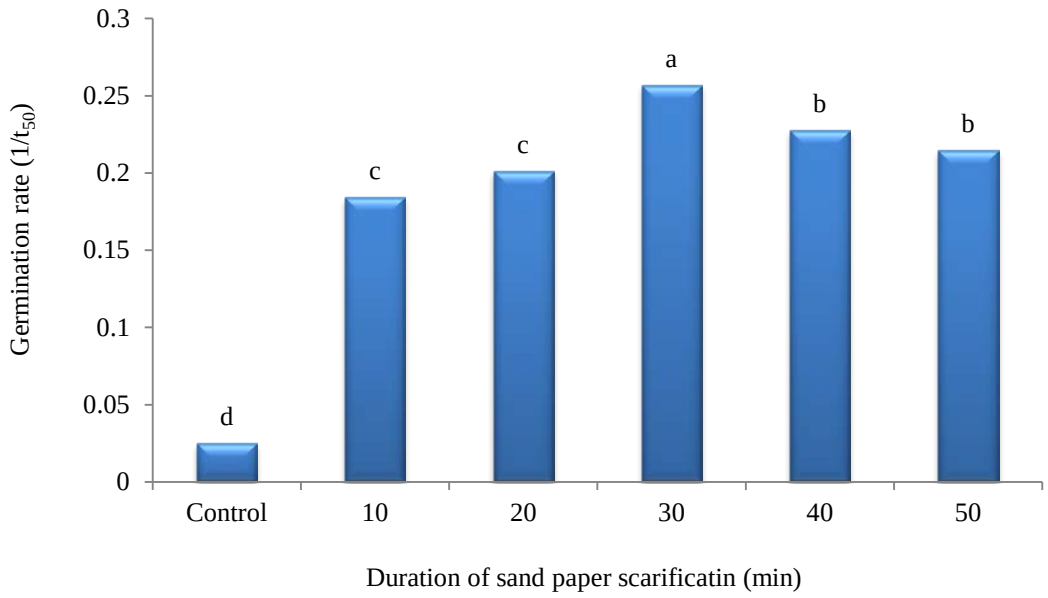

Figure 3.8: Effect of sand paper treatment on germination rate. Means without a letter in common differs significantly at $p \leq 0.05$ using Tukey's test.

It appears from the results that all treatments led to an increase of both germination percentage and germination rate compared with controls. The fastest germination was generally with cut seeds. Treatment with sulphuric acid and sand paper scarified seeds started after only 3 days. On the other hand germination obtained with boiling water treated seeds began only after 15 days.

3.3.2.5 Germination percentage of seed size

The statistical analysis of variance revealed that the germination level (%) of large and medium size seeds was significantly greater than that of small ones (Table 3.1).

Table 3.1: Effect of frequency of boiling water treatment on germination of large, medium and small seeds after 35 days

	Germination (%)		
	Seed size		
Number of boiling water treatments	Small	Medium	Large
0	5 [e]	15 [d]	25 [d]
1	20 [d]	30 [c]	40 [c]
2	25 [d]	45 [c]	60 [b]
3	30 [c]	85 [a]	95 [a]
4	30 [c]	70 [b]	90 [a]

Means not followed by the same postscript letter differ significantly at $p \leq 0.05$ using Tukey's Multi- range test.

Figure 3.9 shows that with the control treatment (cold water only) large seeds started to germinate after 12 days. After 1 month, 25% of large, 15% of medium and only 5% of small seeds had germinated (Table 3.1).

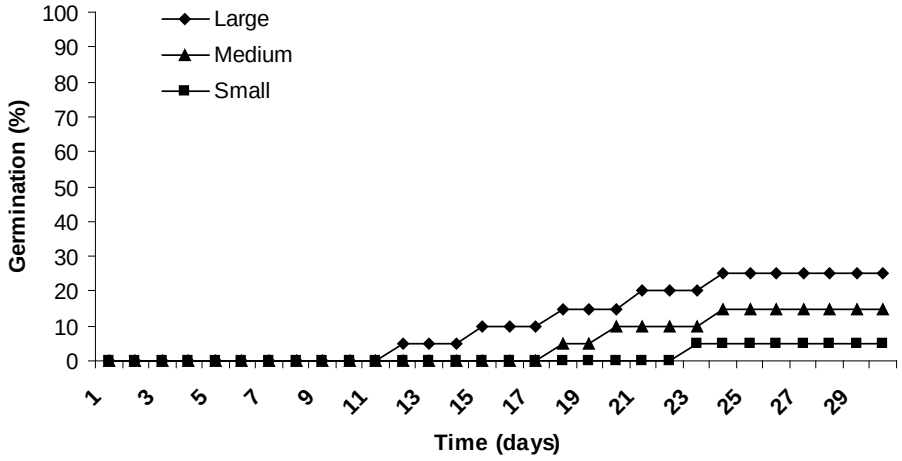

Figure 3.9: Effect of seed size on germination of *Acacia saligna* seeds treated with cold water

To a certain extent *Acacia* seeds treated once with boiling water had an improved germination level compared with the control (Figure 3.10, Table 3.1). Germination of both large and medium seeds was earlier than that of the small ones and germination reached 40%, 30% and 20%, respectively.

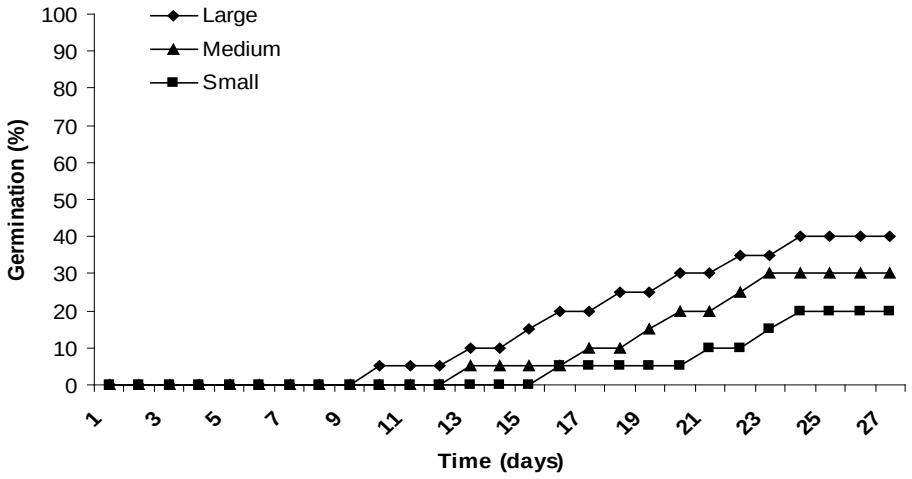

Figure 3.10: Effect of seed size on germination of *Acacia saligna* seeds treated with boiling water once

Treating *Acacia* seeds with boiling water twice increased large seed germination to 60% after 1 months compared with 40% after one boiling water treatment (Figure 3.11, Table 3.1).

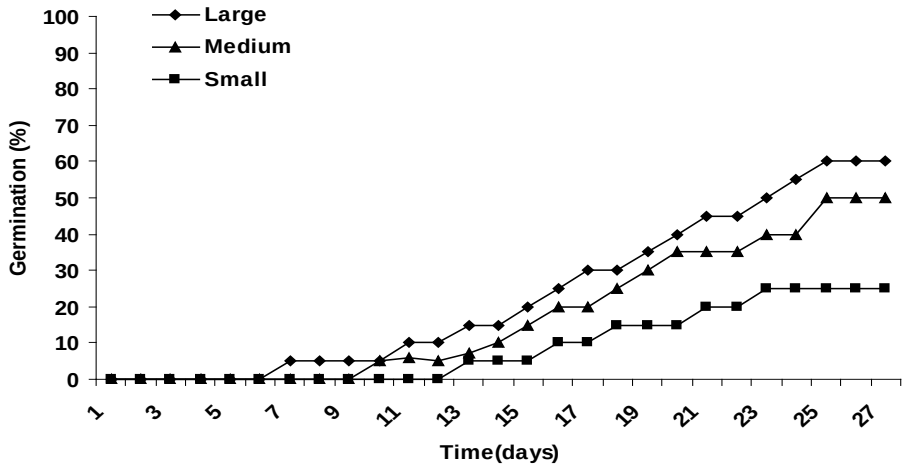

Figure 3.11: Effect of seed size on germination of *Acacia saligna* seeds treated with boiling water twice

Increasing the number of boiling water treatments to three substantially reduced the time to the onset of germination and led to 95% of large seeds germinating after approximately one month (Figure 3.12, Table 3.1).

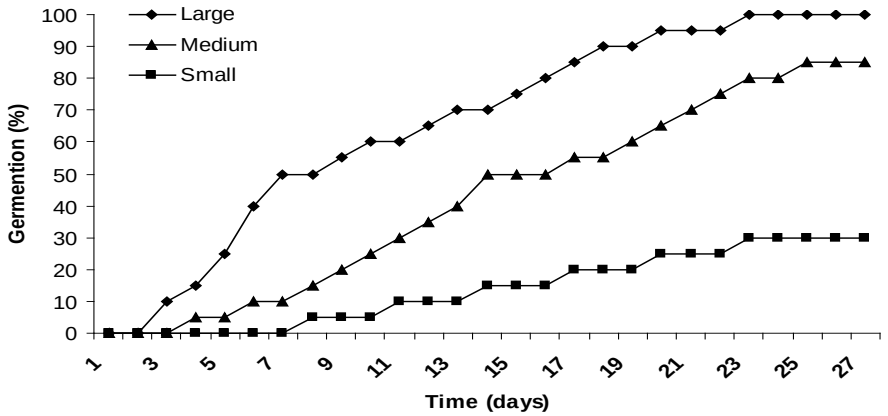

Figure 3.12: Effect of seed size on germination of *Acacia saligna* seeds treated with boiling water three times

ANOVA revealed that there is no significant difference between treating *Acacia* seeds with boiling water four times and three times except for medium seed size, where the germination was significantly lower with four than with three times boiling water treatments (Figure 3.13, Table 3.1).

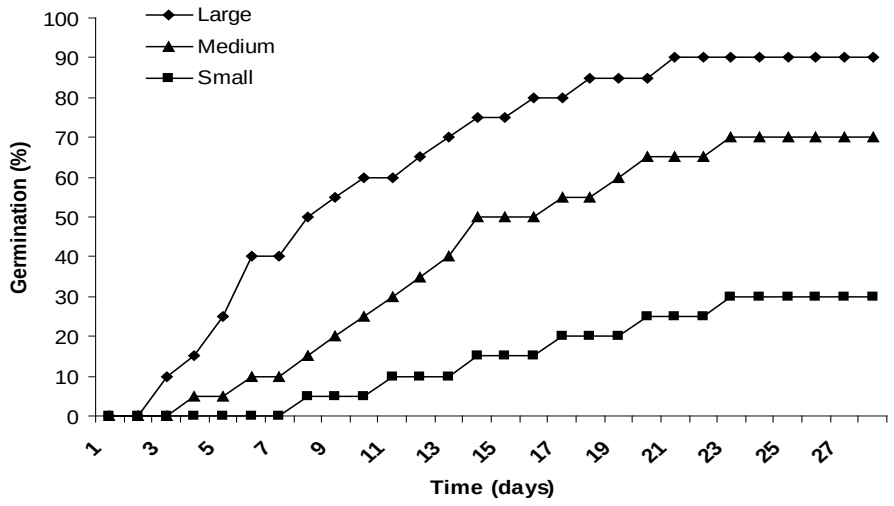

Figure 3.13: Effect of seed size on germination of *Acacia saligna* seeds treated with boiling water four times

3.3.2.6 Germination rate of seed size

The germination rate of seeds increased significantly with boiling water (once, twice, and three times). Significant differences were observed between seedlings from large and small seeds of *A. saligna* with boiling water treatment at boiling once, twice, three, and four times. The rate of germination showed differences between large, medium and small. However the rate of germination was significantly influenced by seed size since the bigger the seed size, the higher the germination rate (Figures 3.14, 3.15).

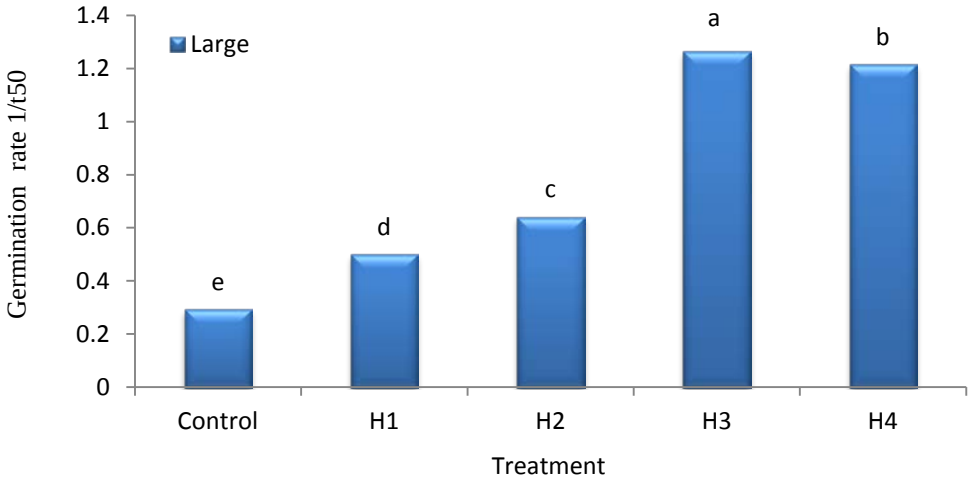

Figure 3.14: Effect of boiling water treatment on germination rate of large seeds. Means without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

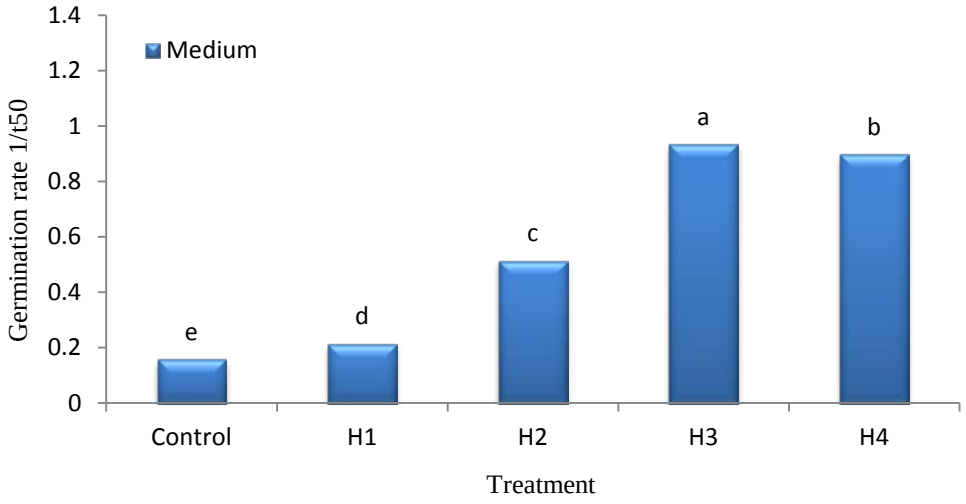

Figure 3.15: Effect of boiling water treatment on germination rate of medium seeds. Means without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

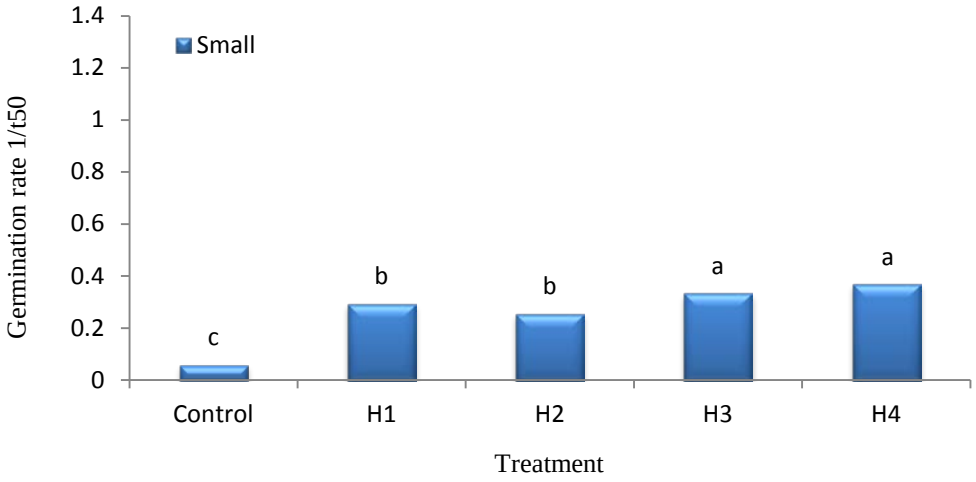

Figure 3.16: Effect of boiling water treatment on germination rate of small seeds. Means without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

3.3.2.7 Effect of sowing depth

Comparing large and medium seeds, there was no significant difference in length of shoots obtained at 20 and 30 mm sowing depth (Table 3.2), but at 10 mm shoot length was significantly greater with large than with medium seeds. Shoot length of large and medium seeds was significantly higher when seeds were sown at 30 mm, followed by 20 mm and then 10 mm. On the other hand shoots of seedlings from small seeds were significantly longer when the seeds were sown at 20 mm depth compared with 10 or 30 mm. There was no germination for seeds of any size with a sowing depth of 40 mm.

Table 3.2: Effect of seed size on length of shoots of seedlings sown at 10, 20, 30 or 40 mm depth

	Shoot length (cm)			
Seed size	10 mm	20 mm	30 mm	40 mm
Large	2.52 [b]	2.94 [b]	3.46 [a]	*
Medium	1.12 [c]	2.90 [b]	3.89 [a]	*
Small	1.71 [c]	2.59 [b]	0.61 [d]	*

Means for shoot length not followed by the same letter differ significantly at $p \leq 0.05$ using Tukey's test.* Seeds of all sizes sown at 40 mm depth did not emerge.

The roots of seedlings from large and medium seeds were significantly longer than those from small seeds when sown at 30 mm, but significantly shorter when sown at 10 mm (Table 3.3). At 20 mm sowing depth there was no significant difference in root length between seeds. It is noticeable that in the case of small seeds sown at 30 mm depth, the root/shoot ratio was about 4 and this was significantly higher than with equivalent seeds at sowing depth of 10 and 20 mm and significantly higher than that with large and medium seeds at the same sowing depth. At a sowing depth of 40 mm there was no germination of seeds of any size (Table 3.4). Increased depth of sowing might increase root/ shoot ratio of small seeds but this advantage could be offset by inferior aeration, and increased mechanical obstruction and the possibility of increased microbial attack on longer hypocotyls.

Table 3.3: Effect of seed size on length of roots of seedlings sown at 10, 20, 30 or 40 mm depth

	Root length (cm)			
Seed size	10 mm	20 mm	30 mm	40 mm
Large	2.88 [b]	3.61 [a]	3.69 [a]	*
Medium	2.35 [b]	3.57 [a]	3.51 [a]	*
Small	3.76 [a]	3.66 [a]	2.76 [b]	*

Means for root length not followed by the same letter differ significantly at $p \leq 0.05$ using Tukey's test.* Seeds of all sizes sown at 40 mm depth did not emerge.

Table 3.4: Effect of seed size on the root/ shoot ratio of seedlings sown at 10, 20, 30 or 40 mm depth

	Root/ Shoot			
Seed size	10 mm	20 mm	30 mm	40 mm
Large	1.14 [d]	1.22 [d]	0.59 [f]	*
Medium	2.09 [b]	1.23 [d]	0.90 [e]	*
Small	2.20 [b]	1.41 [c]	4.52 [a]	*

Means for root / shoot not followed by the same letter differ significantly at $p \leq 0.05$ using Tukey's Test. *Seeds of all sizes sown at 40 mm depth did not emerge.

For large and medium seeds, dry weight of shoots of seedlings was significantly greater at 20 and 30 than at 10 mm sowing depth (Table 3.5), but with small seeds significantly greater shoot dry weight was obtained with sowing at 20 than at 10 or 30 mm depth. At all sowing depths, large seeds produced seedlings with both root (Table 3.6) and shoot (Table 3.5) dry weights significantly higher than those from small seeds. Seeds of all sizes sown at 40 mm depth failed to emerge (Table 3.5). The status of the seeds was checked at the end of a given experiment, to see what happened to those which did not result in established seedlings. It was found that 65% of small, 40% of medium and 10% of large seeds germinated but did not emerge due to the effect of sowing depth.

Table 3.5: Effect of seed size on shoot dry weight of seedlings sown at 10, 20, 30 or 40 mm depth

	Shoot dry weight (g pot^{-1})			
Seed size	10 mm	20 mm	30 mm	40 mm
Large	0.52 [b]	0.81 [a]	0.94 [a]	*
Medium	0.38 [c]	0.48 [b]	0.58 [b]	*
Small	0.38 [c]	0.59 [b]	0.25 [d]	*

Means for shoot not followed by the same letter differ significantly at $p \leq 0.05$ according to Tukey's multiple range tests. *Seeds of all sizes sown at 40 mm depth did not emerge.

Table 3.6: Effect of seed size on root dry weight of seedlings sown at 10, 20, 30 or 40 mm depth

	Root dry weight (g pot^{-1})			
Seed size	10 mm	20 mm	30 mm	40 mm
Large	0.056 [b]	0.088 [a]	0.087 [a]	*
Medium	0.040 [c]	0.054 [b]	0.059 [b]	*
Small	0.040 [c]	0.054 [b]	0.059 [b]	*

Means for root not followed by the same letter differ significantly at $p \leq 0.05$ according to Tukey's multiple range tes.t. *Seeds of all sizes sown at 40 mm depth did not emerge.

When pots were watered with tap water (Figure 3.17, 3.18, 3.19) a small proportion of seeds of each size emerged and survived from 40 mm depth (more from large seeds), but when watered with distilled water (Tables 3.2 and 3.3) no seeds emerged and survived when sown at 40 mm. With large seeds sown at 30 mm survival was significantly higher than with 20 or 10 mm sowing depth (Figure 3.16), while for medium and small seeds at 20 or 30 mm was significantly higher than that at 10 mm depth (Figures 3.17 and 3.18).

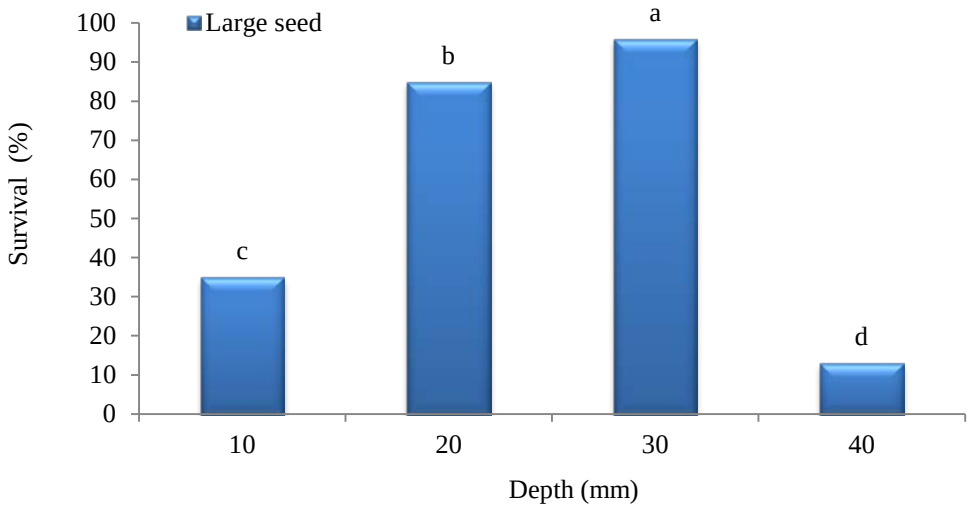

Figure 3.17 Effect of depth of sowing on survival of *Acacia saligna* seedlings from large seeds treated three times with boiling water and sown at 10, 20, 30 or 40 mm. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

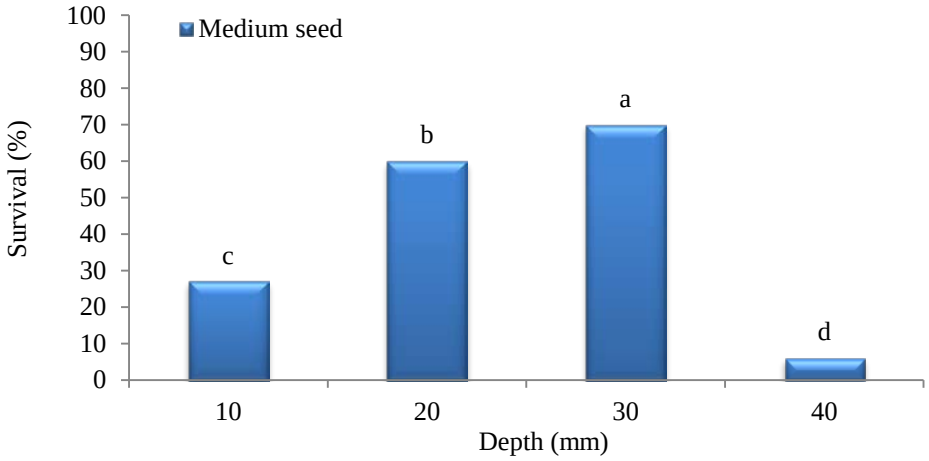

Figure 3.18 Effect of depth of sowing on survival of *Acacia saligna* seedlings from medium seeds treated three times with boiling water and sown at 10, 20, 30 or 40 mm. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

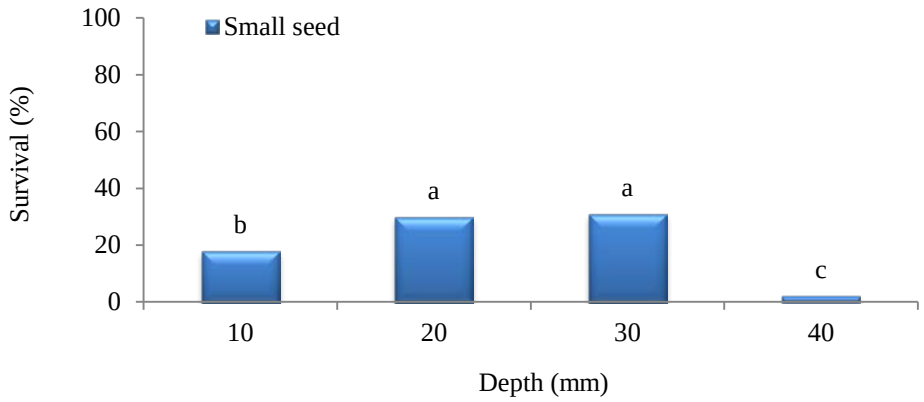

Figure 3.19 Effect of depth of sowing on survival of *Acacia saligna* seedlings from small seeds treated three times with boiling water and sown at 10, 20, 30 or 40 mm. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

Figures 3.20, 3.21, 3.22, 3.23 and 3.24 show the survival of seedlings at 30 mm sowing depth as measured by the number of seedlings which emerged and survived after 7 weeks, the end of the greenhouse experiment. As shown in the laboratory germination test above, survival significantly increased with increasing number of dormancy-breaking boiling water treatments, reaching a maximum with three treatments. With all dormancy breaking treatments, establishment of seedlings was significantly greater from large than from small seeds, with seedling establishment from medium size seeds being significantly lower than from large seeds in the control (cold water) and significantly higher than from the small seeds with one or two boiling water treatments.

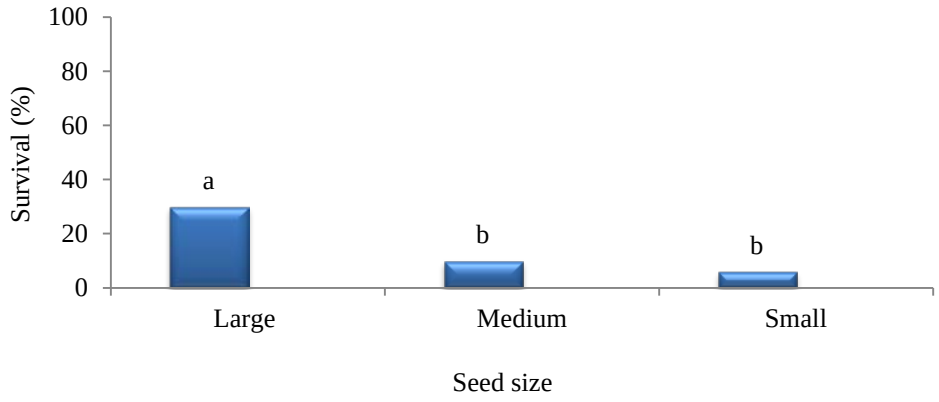

Figure 3.20: Effect of seed size on seedling survival of *Acacia saligna* seedlings from seeds treated with cold water and sown at 30 mm depth. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

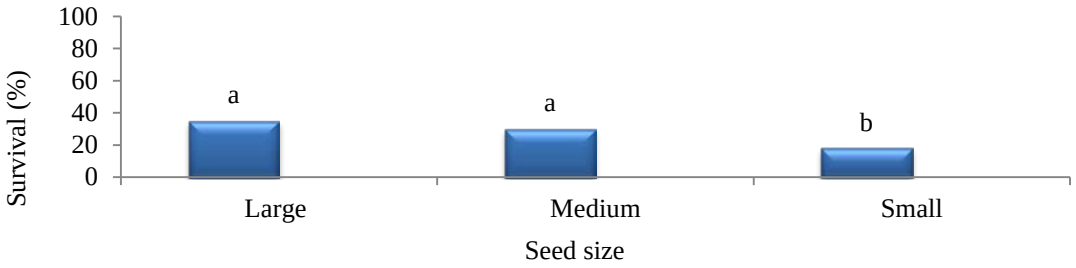

Figure 3.21: Effect of seed size on seedling survival of *Acacia saligna* seedlings from seeds treated with boiling water once sown at 30 mm depth. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

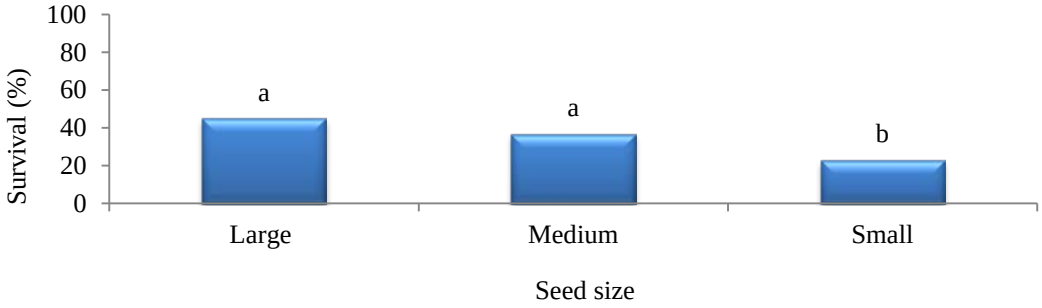

Figure 3.22: Effect of seed size on seedling survival of *Acacia saligna* seedlings from seeds treated with boiling water twice, and sown at 30 mm depth. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

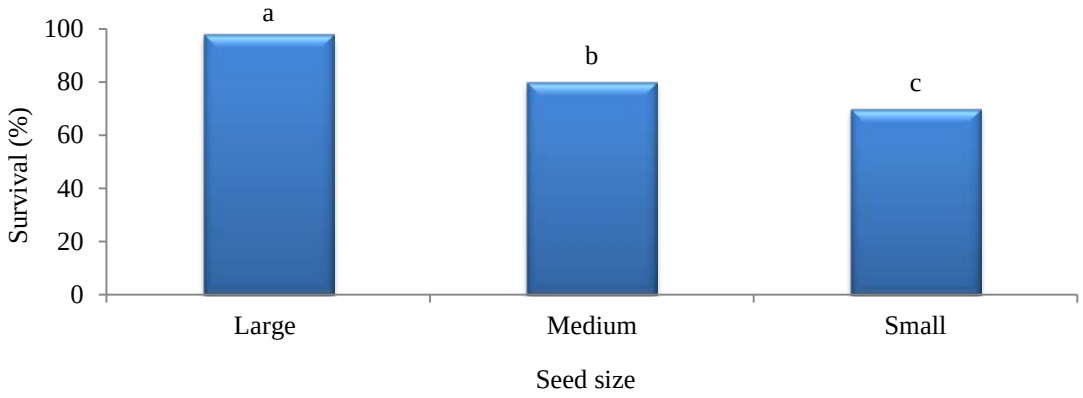

Figure 3.23: Effect of seed size on seedling survival of *Acacia saligna* seedlings from seeds treated with boiling water three times and sown at 30 mm depth. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

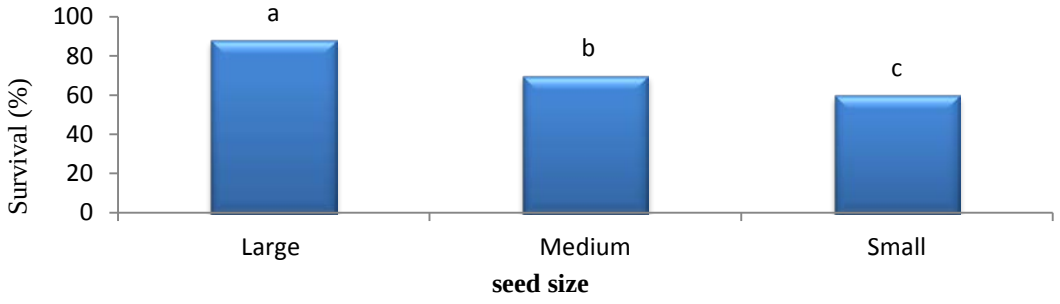

Figure 3.24: Effect of seed size on seedling survival of *Acacia saligna* seedlings treated with boiling water four times and sown at 30 mm depth. Columns without the same letter differ significantly at $p \leq 0.05$ using Tukey's test.

3.4 Discussion

Seeds of *Acacia* species are known to have hard coats which are considered to be one of several strategies for survival in spatially and temporally variable environments. Hard coats can completely prevent the imbibition of water and exchange of gases, thus preventing initiation of the germination process (Khasa 1993). Such physical seed coat dormancy occurs most frequently in species adapted to alternating dry and wet seasons such as that of Northern Libya. To accelerate germination of *A. saligna* seeds, various pre-treatment methods were assayed, including soaking in boiling water, mechanical and chemical scarification of the seed coat. The simplest and most direct physical method is to cut, drill or file a small hole in the seed coat. This has been found to be successful. Scarification by sand paper is also used to reduce seed coat thickness by abrasion, especially on hard coated species. Mechanical scarification is reported to be one of the most effective dormancy breaking treatments of *A. saligna*, but cannot be used on a large scale, notably, the manual treatment of individual seeds. Boiling water scarification treatment had a clear positive impact on germination. Immersion of seeds in boiling water may stimulate germination by causing rupture of the lens tissue, thereby allowing water to enter the seeds as reported by Willian (1985) and Cavanagh (1987). The results further indicated that treatment of *A. saligna* by boiling water more than three times for a total of 90 min is not worthwhile. Boiling water treatment resulted in a permeable seed coat, yet germination was slower than with cut seed. However, the boiling treatments are easy and it is safe to treat large number of seeds in this way. Although treatment by clipping the seed or sand paper abrasion was also successful and gave high germination level and rate, it is regarded as somewhat impractical with a large amount of seed. The results in this experiment were comparable to those of Youssef, Heikal, and Shaker (1991), who found that *A. saligna* gave the highest germination after

boiling water treatment. Khasa (1993) investigated different methods to overcome seed coat dormancy of *A. auriculiformis.* Of the water pre-treatments tested, soaking seeds in boiling water (heat source removed) gave the best germination (77.5% after 20 days of germination). Immersing the seeds in boiling water for 1 min gave the second highest result for water pre-treatments (51.0%). Doran and Gunn (1987) reported *A. saligna* should be scarified by mechanical abrasion or immersion in undiluted sulphuric acid (95%) for 30 min and then thoroughly washed in water. Alternatively, immersion in hot water (90°C) for 1 min will usually break dormancy. Larsen (1964) reported large increases in germination rates after the following procedure: seeds were dropped into ten times their volume of heated water for 30 min, and then immersed in 20 times their volume of cold water, where they imbibed for 18 h. Ninety-one percent of seeds of *A. mangium* pre-treated with boiling water for 30 min germinated. In laboratory trials in Sweden, sandpaper scarification followed by a 3 h cold water soak was the most effective treatment for *A. farnesiana* (Baskin and Cordell 2004). Using sulphuric acid as a seed coat softener, on the other hand, would be difficult in nursery conditions and is a hazardous method, treatment of seeds with acid for 90 min decreased germination level. This may be ascribed to the harmful impact of the acid during this longe period which may lead to destroying a great pat of seed coat and endosperm, consequently led o killing the embryo. The germination rate of the seed of *A. saligna* was also improved by treatment with boiling water in agreement with the results of Larsen (1964) who found that treatment of *A. salgna* with boiling water improved germination rate. The results obtained from the present experiment that seed *A. saligna* displayed physical dormancy due to hardness of their coat. Large seeds germinate earlier and achieve greater final germination than medium and small seeds, and this has been found in many species. Examples include pines, e.g. *Pinus radiata* and *P. toeda.* In the present study there were

large differences in the dry matter accumulated in seedlings developed from large and small seed-size classes and sowing depth. It is generally agreed that large seeds tend to produce larger seedlings (Schaal 1980).

Patterns of absolute growth rate of seedlings have shown that consistently greater absolute growth of seedlings from large seeds than from small seeds is maintained until maturity (Schaal 1980, Stanton 1984) although the initial size advantage of seedlings from large seeds may disappear with time because of higher relative growth rate of seedlings from small seeds (Westoby, Jurado, and Leisman 1996, Bonfil 1998, Khurana and Singh 2000).

There was no significant difference in the dry weight of roots and shoots of seedlings from large and medium seeds sown at 20 and 30 mm depth. However, when small seeds were used, root and shoot dry weights were significantly decreased by deeper sowing.

Shaukat, Siddiqui, and Aziz (1999) suggested that both germination and vigour of seedlings are directly associated with seed size, as seedling vigour was decreased by increasing the planting depth of small *A. nilotica* seeds and conversely, a higher percentage of vigorous seedlings resulted when large seeds were planted deep. For this reason Bolton (1983) and Johnson (1983) suggested that a large seeded cereal crops should be seeded at a maximum 5 to 6 cm (Anderson 1974, Johnson 1983).

These advantages could be offset by inferior aeration, increased mechanical obstruction and the possibility of increased microbial attack on longer hypocotyls. In these experiments the greatest seedling growth of small seeds was obtained when seeds were sown near the soil surface and this may reflect a general survival strategy by *Acacia* spp. and suggests that small seeded crops should be seeded at 2.5 cm or less.

3.5 Conclusion

The present experiment emphasizes the necessity of treating acacia seeds before sowing in seedbeds to promote a high germination percentage and to produce uniform seedlings. Germination, as an indicator, may manifest the detrimental impacts of the methods of dormancy breakdown.

Mechanical scarification of seeds by cutting or sand paper abrasion was successful and gave the highest germination percentage and germination rate. However, it is difficult to replicate this treatment on a large number of seeds due to the creditable time and effect unavoided.

The most applicable methods for overcoming the mechanical dormancy are soaking in H_2SO_4. Treated of *A. saligna* with sulphuric acid can significantly increase seed germination and germination rate. Since sulphuric acid is caustic and dangerous to handle, its use is recommended only for those familiar with the use of caustic chemicals.

Soaking in boiling water is the best suited for larger seed lats before sowing. Soaking in boiling water seems to be the most suitable treatment for *A. saligna.* It is easy and safe to treat large number of seeds in this way.

Treatment of *A. saligna* seeds of all sizes with boiling water three times gave the greatest seed germination. Large seeds germinated earlier and achieved greater germination than small and medium seeds.

The depth, at which seeds are sown, may be a significant factor in assessing *A.saligna* development for management. Sowing *A. saligna* seeds at 30 mm depth gave greatest seedling growth from large and medium seeds whereas 20 mm was more suitable for small seeds.

The greatest seedling growth of small seeds was obtained when seeds were sown near the soil surface (10-20 mm) and this may reflect a general survival strategy adopted by *A. saligna* growth and suggests that small seeds should be sown at 2.5 cm or less.

The selective importance of the size of *A. saligna* is potentially important only for germination of seeds and early growth of seedlings; seed size effects on seedling growth disappear rapidly, at least under glasshouse conditions.

The effects of seed size were very important for the germination and growth of seedlings and could be of adaptive significance in establishing and maintaining the populations, because seed size is an important biological factor, affecting seed germination, seedling elongation, and growth of *A. saligna* and this promoted by large seeds.

Overall, is recommended that for breaking *A.saligna* seed dormancy and increasing seed germination percentages and germination rate in a short time, the most applicable methods is boiling water applied three times. These findings may be useful as a guide for nursery operations leading to the successful establishment.

Chapter 4

Effect of saline irrigation on establishment of *Acacia saligna*

4.1 Introduction

The successful establishment of young seedling depends on physiological and biochemical factors of the seeds. There is a strong interaction between various environmental factors and seedling growth and intolerance of seeds to these factors may result in physiological and biochemical disturbance. An understanding of responses of plants to salinity is of great practical importance; the presence of saline conditions in the germinating environment may be a critical factor in seed survival and can prevent the plant species from successfully colonizing an area. It has been reported that high concentrations of salts have detrimental effects on plant growth (Kramer 1983, Bernstein, Lauchli, and Silk 1993, Garge and Gupta 1997, Mer *et al*. 2000) and excessive concentrations can kill growing plants (Donahue, Miller, and Shickuna 1983). Many investigators have reported retardation of germination and growth of seedlings at high salinity (Bernstein 1961/2, Garge and Gupta 1997). However, plant species differ in their sensitivity or tolerance of salinity. There are many different types of salts and almost an equally diverse set of mechanisms of avoidance or tolerance. In addition, organs, tissues and cells at different developmental stages of plants exhibit varying degrees of tolerance to environmental conditions (Ashraf 1994). It is known that shoot growth is often suppressed more than root growth by soil salinity (Mass and Hoffman 1977, Ramoliya, Patel, and Pandey 2002). However, there have been relatively few studies on the effect of soil salinity on root growth (Garge and Gupta 1997). The effect of high concentration of salts on the growth of plants is primarily through the soil

solution. Therefore, it is expected that dry soil may effect plant growth more than wet soil. An extended period of drought is almost a regular phenomenon in saline deserts.

The aim of the study presented in this chapter was to establish the effect of saline irrigation on *A. saligna* as a preliminary study before testing treatments to mediate the effect of saline irrigation.

4.2 Materials and methods

The field experiments were carried out in the Experimental Station of the Faculty of Agriculture in the Sidi El Mesri area of Tripoli, Libya from May to July 2007. Environmental data are given in Appendix 1. Greenhouse experiments were done at Coventry University (52° 24' N 1^{o} 31'W) in England. The greenhouse experiment was conducted from January to March 2007, with daylight and supplementary light from 400 W high pressure sodium Son/T Lamps providing a minimum 16 h photoperiod and photon flux of 180-210 μmol m^{-1} S^{-1} at bench level. Minimum day and night temperature were 25- 27 and 15-17 ^{o}C respectively. Relative humidity ranged between 40 and 60%. Sand was used in the greenhouse trial. In the field, the soil was taken from the upper most surface soil horizon found at Sidi El Mesri Tripoli Libya. Tables 4.9 and 4.10 show the characteristics of the medium used in the field experiment. Mean day time temperature was 40 ± 5 ^{o}C. Seeds of *A. saligna* were sown in pots with a minimum diameter of 130 mm. The bottom of the pots contained a piece of filter paper cut to size to prevent the soil falling out when dry. Seeds were soaked by using 100 ml of boiling water poured onto 60 seeds for each treatment and left to cool; boiling water was applied three times for 30 min as identified in Chapter 3. After that the seeds were checked to see if they had imbibed. Thereafter, 10 imbibed seeds were sown in each pot. There were seven treatments including a control using six replicate blocks for each treatment. The experiment was laid

out in a randomized block design to minimize the effect of any environmental difference. Seeds were sown and irrigated with distilled water to field capacity for the first 2 weeks of the experiment and any leachate of water was retained in saucers. In the third week, all the plants except the control were watered with 50 ml of NaCl at 0.5 M or 1.0 M. The 0.5 M and 1.0 M NaCl concentrations tested in this study were chosen as similar to the ground water concentrations of soluble salts found in some soils in Libya. In the regions depending on ground water, the high salt concentration caused by the upward movement of water and a high rate of evapo-transpiration, results in the problem of salinity, especially in the north of Libya where the ground water contains 1500 ppm of soluble salts. By the fourth week, two treatments received a further 50 ml of 0.5 M or 1.0 M NaCl while the rest were watered with distilled water. During the fifth week one treatment received a third lot of 50 ml of 0.5 M NaCl and one 50 ml of 1.0 M NaCl. Table 4.1 shows the watering regime of the plants.

Table 4.1: Irrigation applied on each occasion to pots (D.W= Distled water)

		0.5 M NaCl			1.0 M NaCl		
Times	Control	Once	Twice	Thrice	Once	Twice	Thrice
	(n-6)	(n-6)	(n-6)	(n-6)	(n-6)	(n-6)	(n-6)
WK1	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.
WK2	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.
WK3	D.W.	50 ml	50ml	50ml	50 ml	50 ml	50 ml
WK4	D.W.	D.W.	50 ml	50 ml	D.W.	50 ml	50 ml
WK5	D.W.	D.W.	D.W.	50 ml	D.W.	D.W.	50 ml
WK6	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.
WK7	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest

Pots were irrigated to field capacity and any leachate was retained in the saucers. All plants received 50 ml of Hoagland's nutrient solution (Table 4.2) one week after seeds were sown and again one week later.

Table 4.2: Hoagland's solution used as a fertilizer

Component	Weight (mg)
$MgSO_4$	64.0
$Ca(NO_3)_{2.}H_2O$	118.0
KNO_3	53.0
$FeSO_4.7H_2O$	2.78
Na_2EDTA	3.77
Micronutrient stock	50 ml

The Hoagland's solution includes an iron complex of ethylene tetra acetic acid (FeEDTA) as a starting point. Stock solution contains 5 mg of Fe in each ml. It was made by dissolving 3.73 g Na_2EDTA in 1 l H_2O, and adding 2.78 g $FeSO_4.H_2O$ afterward. The micronutrient stock solution contained 125 mg of H_2BO_3 (boric acid), 125 mg of $MnCl_2.4H_2O$ (manganese chloride), 12.5 mg of $ZnSO_4$ (zinc sulphate), 5.0 mg of $CuSO_4$ (copper sulphate) and 14.5 mg of $Na_2MoO_4.H_2O$ (sodium molybdate) per litre.

4.2.1 Determination of pH and electrical conductivity

For determination of pH 10 ml (a scoop containing 10 ml of sand filled and struck off level without tapping) aliquots of air-dried soil, ground to pass through a 2 mm mesh sieve were transferred into a plastic bottle to which was added 20 ml of distilled water

using a graduated cylinder. The plastic bottle was mixed well with a glass rod and shaken on a shaker for 15 min. The suspension was filtered through a 125 mm Whatman No.2 filter paper. The filtrate was used to determine the pH using a pH meter. The pH meter was calibrated using buffer solutions of pH 4.0 and 7.0. The soil suspensions were stirred with a glass rod, the electrodes inserted about 3 cm deep and the reading taken after 30 s (Bailey 1990). After this, the combined electrode was removed from the suspension, and rinsed thoroughly with distilled water in a separate beaker. For conductivity, 10 ml scoop samples of air-dried growth medium were transferred to a plastic poly bottle to which was added 25 ml of deionized water. The bottle was capped and than shaken for 15 min on a reciprocating shaker. The suspension was filtered through a 125 mm Whatman No. 2 filter paper and the filtrate was used to determine the electrical conductivity.

4.2.2 Determination of particle size distribution

Mechanical analysis for sand, silt and clay particles was carried out using a standard hydrometer, ASTM no. 152, as described by Black, Evans, and White (1965). Air-dry soil samples (50 g) were weighed into a 200 ml beaker. Each soil sample was wetted by means of a small amount of distilled water. Twenty ml of 5% calgon solution 50 g calgon Na-hex-metaphosphate 1^{-1} deionised water (McElreath and Johnson 1990) was added to the soil sample. The contents were mixed, covered with a watch glass and kept overnight. The contents were quantitatively transferred into a dispersion cup using a stream of distilled water and mixed in an electrical mixer for 5 min. The suspension was than transferred into a 1 L sedimentation cylinder with the help of a stream of water and made up to the mark with distilled water. The suspension was allowed to equilibrate thermally, and then its temperature was recorded. A plunger was inserted, and moved up and down to mix the contents thoroughly for 1 min, carefully holding the bottom of the

cylinder to prevent tipping. The stirring was finished with two or three slow, smooth strokes, the plunger was removed tapped slightly to remove adhering drops and amyl alcohol was added to disperse the foam. After 20 s, the hydrometer was carefully lowered into the suspension and the reading at the top the meniscus was taken after exactly 40 s. This reading gave the amount of the silt and clay. Care was taken to ensure that the hydrometer was kept away from the cylinder wall. The hydrometer was then removed, rinsed and dried with tissue paper. The suspension was allowed to stand for 2 h (including the 40 s reading) then the hydrometer was again inserted very carefully and its reading was recorded followed by a temperature reading. This reading gave the amount of clay present. The hydrometer type used (ASTM no.152) was calibrated at 19.5^{o}C. A temperature correction of 0.3 graduations on the hydrometer for every 1^{o}C above or below 19.5^{o}C was applied.

Calculation:

The first reading (at 40 s) gave the amount of silt and clay. The second reading (after 2 h) gave the amount of clay. The amounts were calculated as following:

$$\% \text{ (silt + clay)} = \frac{\text{Corrected hydrometer reading (40 s)}}{\text{Oven-dry soil weight (g)}} \qquad \text{(Equation 4.1)}$$

$$\% \text{ Clay} = \frac{\text{Corrected hydrometer reading (2 h)}}{\text{Oven-dry soil weight (g)}} \qquad \text{(Equation 4.2)}$$

$$\% \text{ silt} = \% \text{ (salt + clay)} - \% \text{ clay} \quad \text{and} \quad \% \text{ sand} = 100 - \% \text{ (silt + clay)}$$

4.2.3 Determination of total nitrogen

Soil samples were analysed for total nitrogen using the modified Kjeldahl method supplied by Buchi Laboratories, Switzerland (Buchi Nitrogen Information Leaflet No.1). Five mg of ball-milled soil was transferred into a 250 ml digestion tube, and 12 ml of salicylic acid-sulphuric acid mixture (25 g $HOC_6H_4CO_2H$ dissolved in 1 l of concentrated H_2SO_4) was added, and contents were mixed thoroughly and allowed to stand at room temperature overnight. Approximately 0.5 g of previously ground sodium thiosulphate ($Na_2S_2O_3.5H_2O$) was added in the tubes. These were transferred to a digestion block and heated at 250 ^oC for 2 h using a fume extractor. The heated mixture was removed from the block and allowed to cool. Afterwards two tablets of copper catalyst were added and tubes were heated again on a digestion unit at 400 ^oC for 2 h in a fume extractor. Once cooled, the contents were diluted by the addition of approximately 75 ml distilled water, and NH_4^+ was determined in the digest by steam distillation. Samples were digested on a batch process basis. Each batch consisted of 20 digestion tubes (the capacity of the Tector DS20 heating block) and samples were analysed in duplicate, including two blanks in each batch. Samples were weighed out onto a 5 cm filter paper and transferred with the filters to the digestion tubes, so filter papers were included in the reagent blanks. The steam distillation for this method was carried out with the Kjeltec Auto 1030 Analyzer.

Using an automated steam distillation apparatus, NH_4^+ is converted to NH_3 by treatment with sodium hydroxide, and distilled into an acid-base indicator solution by the delivery steam. An autotitration is performed with standard acid, and the volume of acid required is automatically calculated and displayed by the instrument.

The steam distillation apparatus (Kjeltec Auto 1030 Analyzer Tubes AB, Sweden) was prepared for use in accordance with the manufacturer's instructions, using 10 M NaOH, 0.1 M HCl (concentration known to four decimal place). A distillation receiver containing 60 ml 2% boric acid (2 g 100 ml^{-1} distilled water) and five drops of indicator in a 250 ml digestion tube was placed in position in the distillation unit, and the safety door closed. Distillation and titration of NH_3 was performed automatically, and the volume of standard acid used (in ml) was displayed. The total N concentration of digested sample was calculated using equation 5.3. The method is derived from Bremner and Mulvaney (1982).

Calculation of result

$$\text{Total } N_2\ (\%) = \frac{V1 - V2 * M * 14.01 * 100}{\text{Weight of soil sample (mg)}} \qquad \text{(Equation 4.3)}$$

V_1 is the soil titre (ml HCl)

V_2 is the blank titre (ml HCl)

M is the molarity (ml HCl known to 4 decimal places)

Weight of soil sample in (mg)

4.2.4 Soil total organic content (% by loss on ignition)

Organic matter in soil was measured by loss in weight on sample ignition (Nelson and Sommers 1996). Three replicates of about 10 g of soil were accurately weighed into crucibles and placed in a muffle furnace and heated at 500 ^oC in an oven overnight and allowed to cool before re- weighing The loss in weight was expressed as % of the initial weight of the soil sample (Equation 4.4).

Calculation

Mass of the soil sample before heating - Mass of the soil sample after heating = Mass of the organic matter.

The percentage of organic matter was determined by the following equation:

$$\% \text{ OM} = \frac{\text{Mass of OM}}{\text{Mass of soil sample before heating}} \times 100 \qquad \text{(Equation 4.4)}$$

4.2.5 Plant parameters

Plant seedling survival was measured weekly before harvesting in the greenhouse in March 2007 and in the field in Libya in July 2007. All plants were harvested from each pot; the plants were harvested carefully by removing them from the pots and washing away soil debris with running water. Plants were then carefully blotted dry with tissue paper and weighed immediately. The plants and number of leaves were counted and divided into root and shoot components using scissors.

4.2.5.1 Fresh weight

Fresh plants were weighed on an electronic balance. Plants were put into plastic bags after harvest to keep them fresh until they could be taken to the laboratory.

4.2.5.2 Dry weight

Plants were removed from plastic bags and placed in an air circulation oven at 80°C for 28 h to dry. Following this they were allowed to cool in desiccators then weighed again for dry weight of roots and shoots.

4.2.5.3 Moisture content of plants

Three replicate samples of fresh plant material from each treatment were placed in foil trays, weighed and dried in the oven at 80 ^{o}C for 48 h, allowed to cool and reweighed. The percentage of water in the sample was calculated by the weight difference using the following equation

$$\text{Moisture of plants \%} = \frac{FW - DW}{FW} \times 100 \qquad \text{(Equation 4.5)}$$

Where FW = Fresh weight DW = Dry weight

4.2.6 Chemical analysis

4.2.6.1 Analysis of plant material

Samples were retained of plants from the saline water irrigation experiments in the greenhouse and in the field. Three replicates were collected from each of the seven treatments; thus there were 21 plant samples. Shoots and roots of the plant samples were dried in an oven at 80^{o} C for 24 h and ground to pass through a 2 mm mesh sieve. Five samples (0.5 g) were placed in plastic bags. The dried shoot and root materials from each plastic bag were digested with nitric acid and the amount of Ca, Na, K, Mg, P and Fe ions determined by atomic emission spectroscopy. Approximately 0.5 g of dry plant

material was weighed into a filter paper (to avoid material losses) and transferred into a digestion tube. Nitric acid (5 ml) was added to the tubes before microwaving for 50 min; the tubes were then cooled for few minutes and distilled water added. The contents of the tubes were filtered into 25 ml volumetric flasks, washing the digest tubes repeatedly with distilled water. Distilled water was added to the volumetric flask to a volume of 25 ml. Each batch of samples contained three controls: nitric acid only, nitric acid + filter paper and nitric acid + 0.5 ml Ca, Na, K, Mg, Fe and P.

4.2.6.2 Soil digestion

Na, Ca, K, P and Fe ion concentrations in soil were estimated by placing 10 g air-dry soil into a 250 ml calibrated digestion tube. Eight ml of concentrated nitric acid was added instead of adding 3 ml 60% perchloric acid to oxidize the organic matter. Perchloric acid presents an additional hazard in that it mists and vapour condenses in ventilation systems to form metallic perchlorates, which can be explosive. Tubes were placed in a block digest and gently heated to 80^{o}C for 2 h and then at 100^{o}C overnight until the solution became clear. The mixture was cooled and filtered through a 125 mm Whatman No. 1 filter paper, the solution was then diluted to 100 ml with deionised water.

4.3 Statistical analysis

Data were statistically analysed using the Minitab 15 Computer Package. The mean separation for all-pairs comparisons was undertaken using Tukey's test at $p \leq 0.05$. Final survival data was arcsin transformed before analysis.

4.4 Results

4.4.1 Results in greenhouse

4.4.1.1 Seedling survival

Figure 4.1 shows the decline of seedling survival with time as they were watered with saline water. Emergence was initially greater than 90% in all treatments.

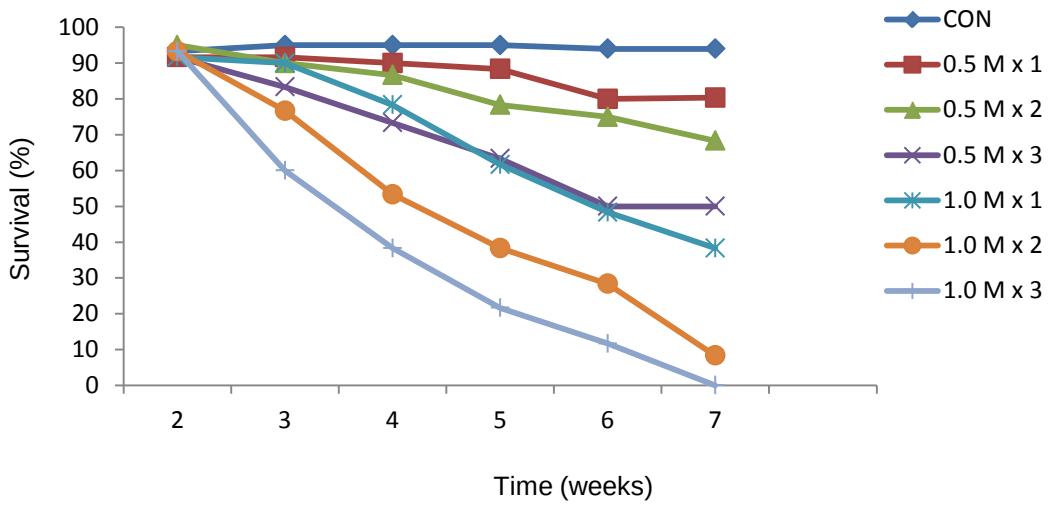

Figure 4.1: Effect of frequency and concentration of NaCl irrigation on survival of *Acacia saligna* in the greenhouse,

4.4.1.2 Shoot fresh and dry weight

Figure 4.2 shows that increasing the concentration of salt in the soil significantly reduced plant growth so that shoot fresh weight significantly decreased with increased frequency of NaCl irrigation. Treatment with 1.0 M NaCl more than once had a greater effect than treatment with 0.5 M NaCl particularly when applied more than once.

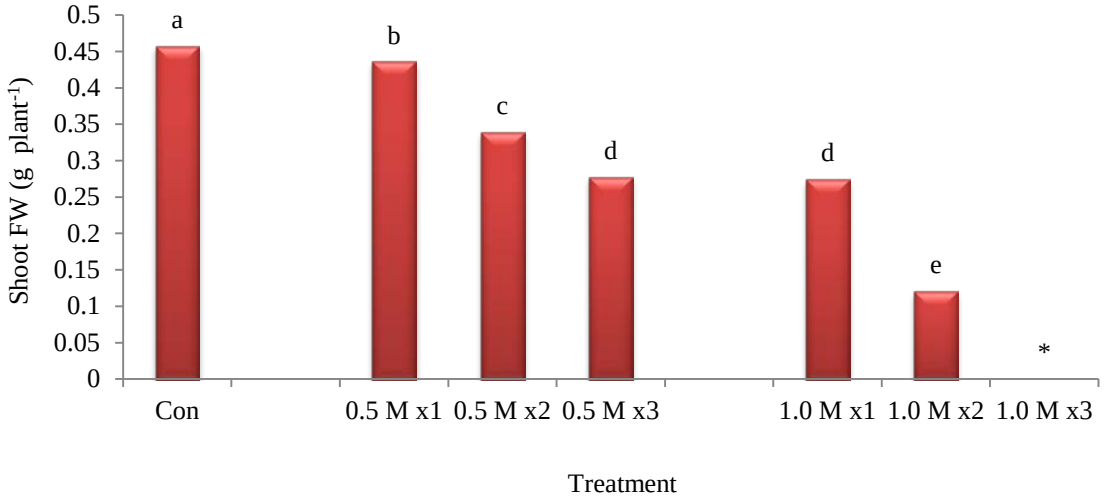

Figure 4.2: Effect of salinity on shoot fresh weight of surviving plants after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance using Tukey's test. * All plants died.

Figure 4.3 shows that there was a significant negative effect of saline irrigation on plant dry weight. Shoot dry weight was significantly reduced with 1.0 M NaCl applied once or more and with 0.5 M NaCl applied three times.

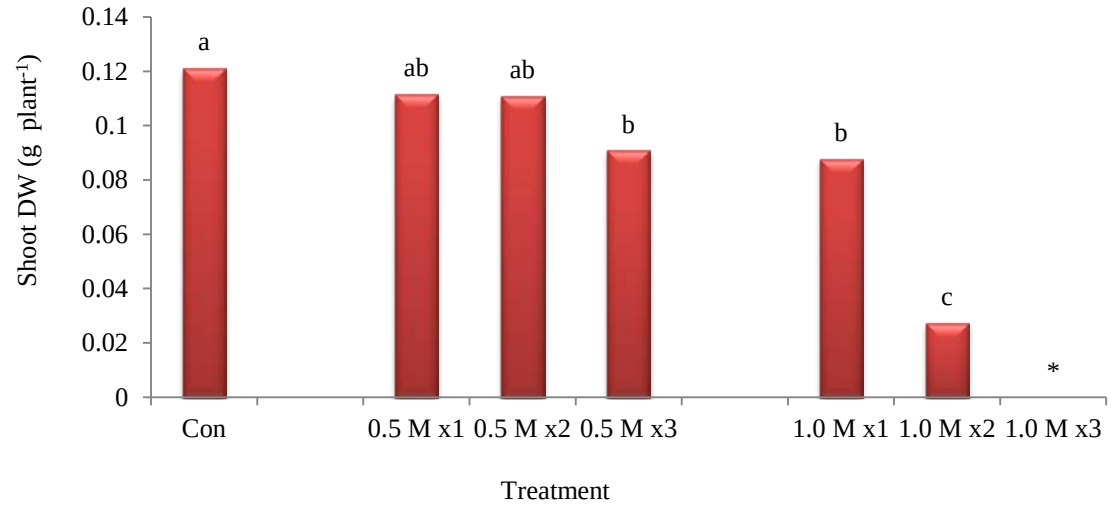

Figure 4.3: Effect of salinity on shoot dry weight of surviving plants after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance using Tukey's test. * All plants died.

4.4.1.3 Shoot moisture content

The shoot moisture content decreased significantly with increasing salinity (Figure 4.4). However, there were no significant difference in shoot moisture content between plants treated with 0.5 M NaCl or once with 1.0 M NaCl, or between the moisture content of plants treated once or twice with 0.5 M NaCl and the control.

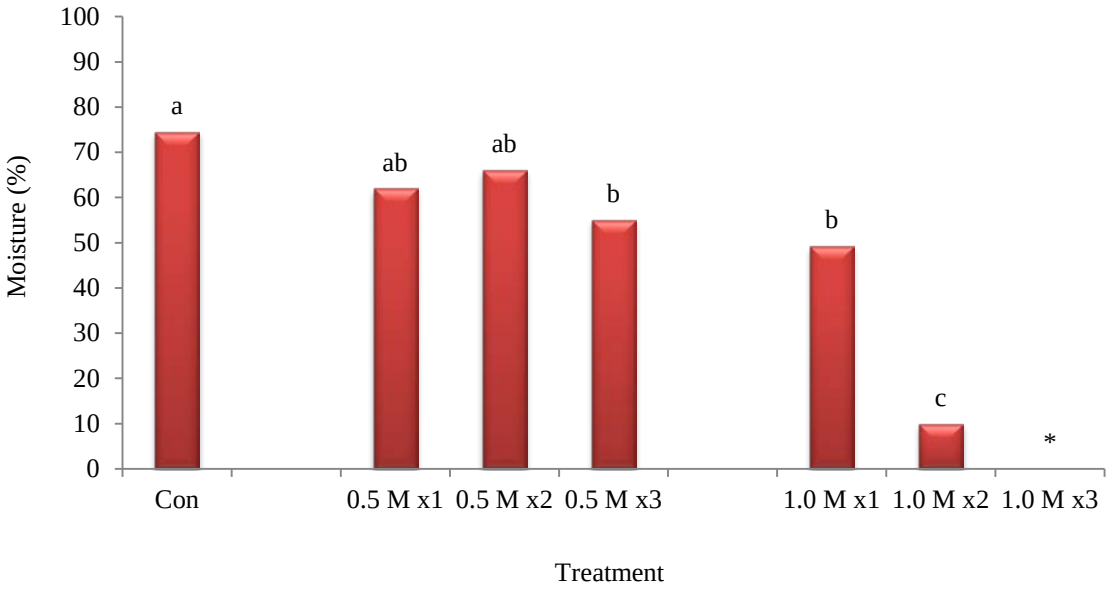

Figure 4.4: Effect of salinity on shoot moisture content of surviving plants after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance using Tukey's test. * All plants died.

4.4.1.4 Root fresh and dry weight

Figure 4.5 shows that root fresh weight of *A. saligna* seedlings irrigated with NaCl decreased substantially with increasing salinity level. The decrease was significant compared to control plants, with three application of 0.5 M NaCl and with one or more application of 1 M NaCl.

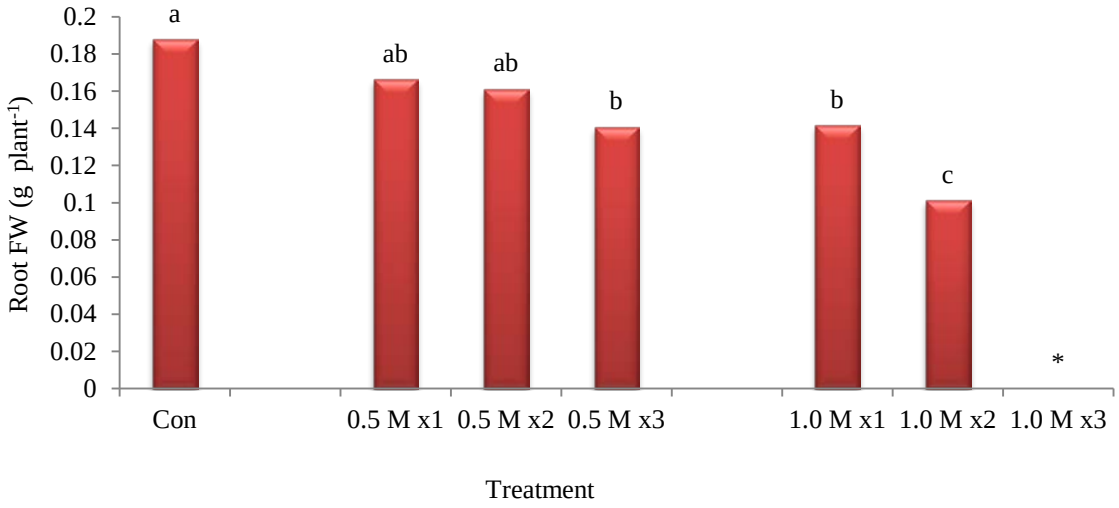

Figure 4.5: Effect of salinity on root fresh weight surviving plants after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Figure 4.6 shows that the frequency of irrigation with 1.0 M NaCl had a significant effect on root dry weight. Dry weight of root was significantly reduced with two applications of 1.0 M NaCl irrigation but not with once. However irrigation with 0.5 M NaCl did not make a significant difference to root dry weight compared with the control, regardless of the frequency of application.

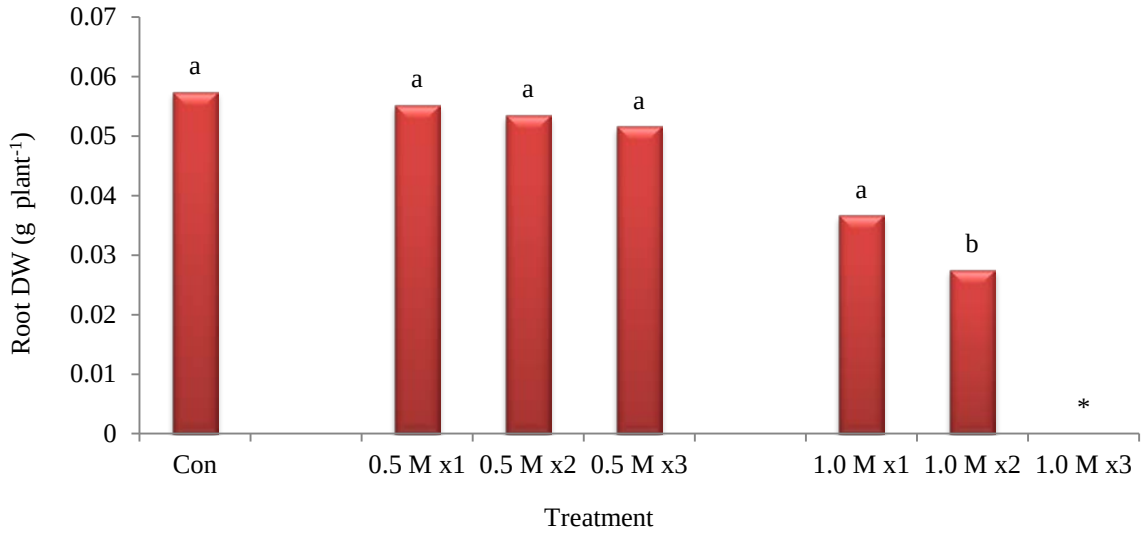

Figure 4.6: Effect of salinity on root dry weight surviving plants after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

4.4.1.5 Root moisture content

Figure 4.7 shows that the root moisture content of plants displayed significant variation with both salt concentration and frequency of irrigation with 1.0 M NaCl. Moisture content of the seedlings was significantly lower with 2 x 1.0 M NaCl application compared with the control. However, a single application of 1.0 M NaCl or any frequency of application of 0.5 M NaCl did not significantly alter root moisture content.

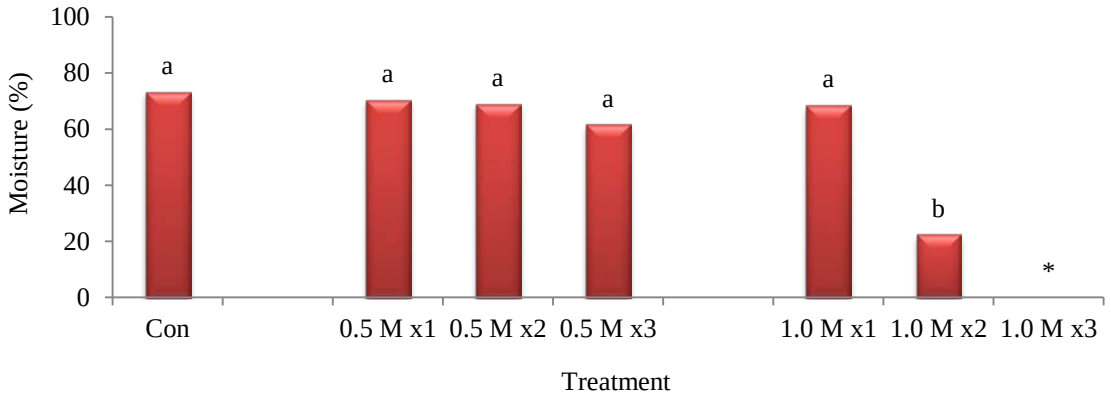

Figure 4.7: Effect of salinity on root moisture content of surviving plants after 7 weeks in the greenhouse Means .without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance using Tukey's test. * All plants died.

4.4.1.6 Root/Shoot ratio

Figure 4.8 show that root/shoot ratio was significantly increased with 0.5 M NaCl at all frequency of application and with 0.5 M NaCl or 1.0 M NaCl once but significantly reduced by irrigation with 1.0 M NaCl twice.

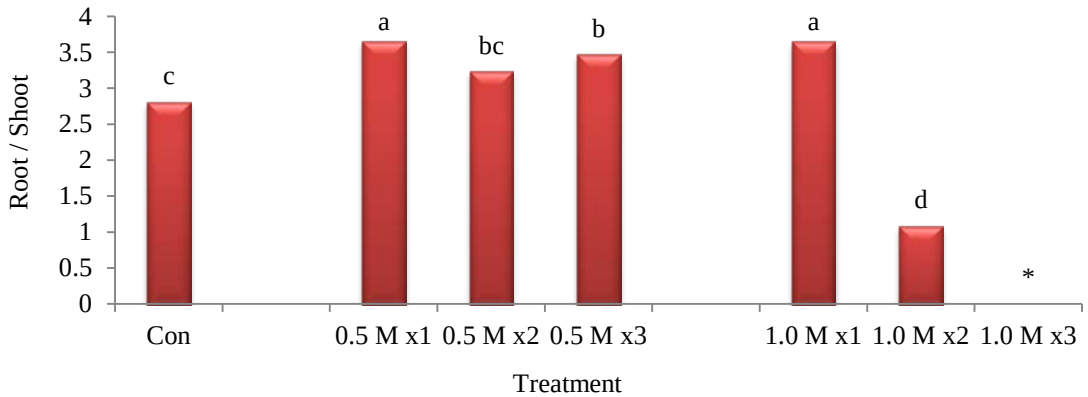

Figure 4.8: Effect of salinity on root/ shoot ratio of surviving plants after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

4.4.1.7 Shoot height and leaf number

Figure 4.9 shows that shoot height was substantially decreased by NaCl applied at both concentrations.

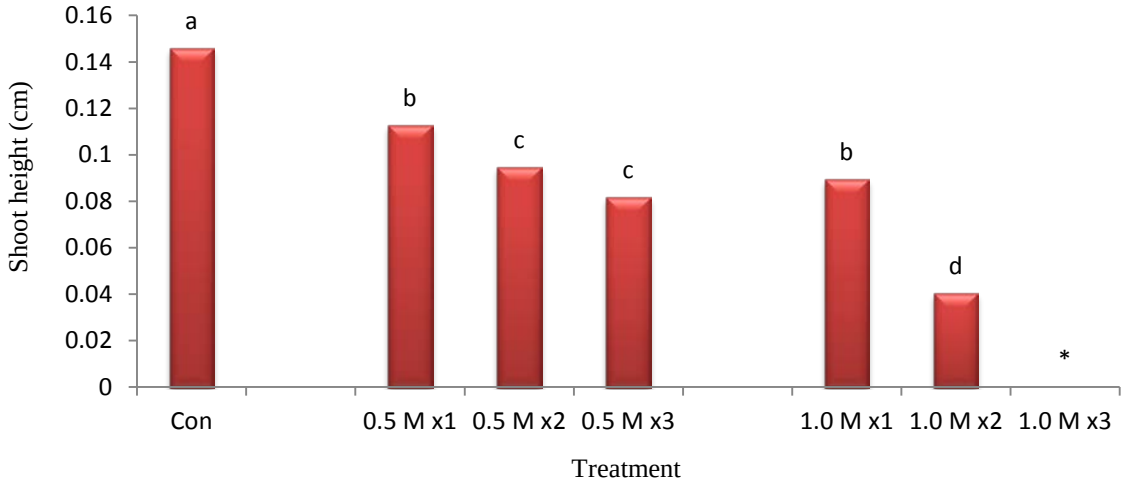

Figure 4.9: Effect of salinity on plant height after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two- way analysis of variance using Tukey's test. * All plants died.

Figure 4.10 shows that the number of leaves decreased with increased frequency and concentration of NaCl irrigation.

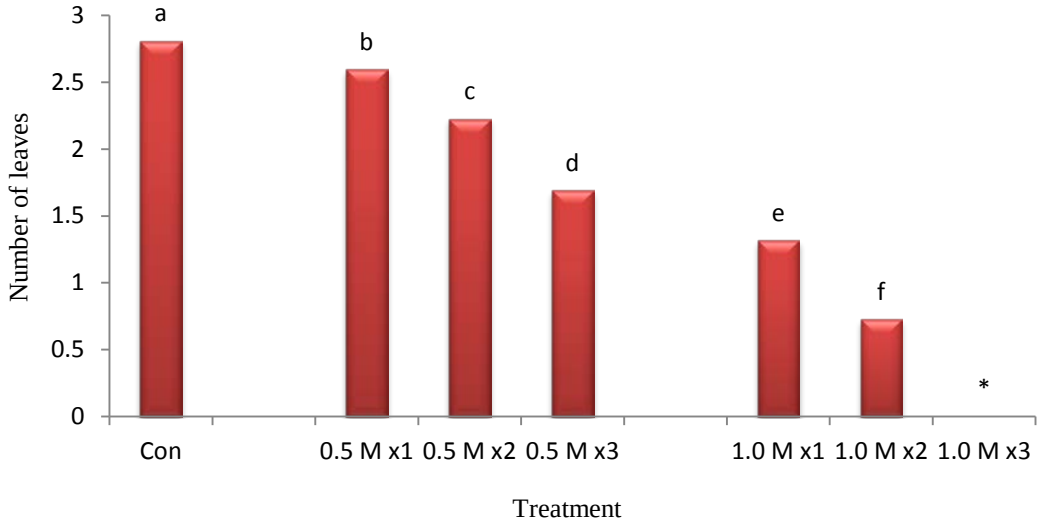

Figure 4.10: Effect of salinity on number of leaves after 7 weeks in the greenhouse. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

4.4.1.8 Mineral content of the medium in the greenhouse

Tables 4.3 and 4.4 show a summary of physical and chemical properties of the sand used in the greenhouse at the beginning of experiment.

Table 4.3: Chemical and physical properties of the medium used in the greenhouse at the beginning of the experiment (values are means of three replicate measurements)

Sand (%)	91.0
Silt (%)	7.4
Clay (%)	1.6
Texture	Sand

Table 4.4: Mean chemical properties of the medium used in the greenhouse at the beginning of the experiment (values are mean of three replicate)

Chemical and physical properties	
pH	4.6 - 4.8
EC (dS m^{-1})	2.25-2.27
OM (%)	0.028
Total nitrogen (%)	< 0.022
Elements	Concentration (mg kg^{-1})
K	97.41
Ca	1532.4
Na	10.75
Mg	358.9
P	86.78
Fe	136.0

Concentrations of calcium, potassium, sodium, magnesium and phosphorus in the medium at the end of greenhouse experiment are presented in Table 4.5. Irrigation with NaCl resulted in significantly increased accumulation of Na^+ compared with the control. The amount of Na^+ increased significantly with increased concentration and frequency of NaCl irrigation even at low levels of salinity. Salinity resulted in significantly increased concentration of K^+ with 1.0 M NaCl compared to the non-saline control. The amount of both Ca^{++} and Fe^{++} was significantly higher with all frequencies of saline application at both 0.5 and 1.0 M NaCl concentrations, compared to the non-saline controls. For Mg^{++}, irrigation with 0.5 M NaCl produced no significant change in Mg^{++} concentration but there was a significant increase when 1.0 M NaCl was applied. Application of both 0.5 M and 1.0 M NaCl treatments at all frequencies significantly increased P compared to control but there was no different between NaCl treatments.

Table 4.5: Concentration of potassium, calcium, sodium, magnesium, iron and phosphorus in medium in the greenhouse at the end of experiment

NaCl treatment	Concentration (mg kg^{-1})					
	K	Ca	Na	Mg	Fe	P
Control	156.2 ^{b}	1606.1^{c}	11.2 ^{g}	490.5 ^{b}	33.5 ^{b}	78.1^{b}
0.5 M x 1	158.7 ^{b}	1607.6 ^{b}	230.7 ^{f}	491.0 ^{b}	34.6 ^{a}	79.5 ^{a}
0.5 M x 2	159.4 ^{b}	1608.2 ^{b}	381.2 ^{e}	491.3 ^{b}	34.7 ^{a}	79.7 ^{a}
0.5 M x 3	161.3 ^{a}	1608.4 ^{b}	524.9 ^{c}	491.4 ^{b}	34.8 ^{a}	79.9 ^{a}
1.0 M x 1	163.0 ^{a}	1610.5 ^{ab}	403.3 ^{d}	491.5 ^{a}	34.9 ^{a}	81.7 ^{a}
1.0 M x 2	165.4 ^{a}	1612.4 ^{a}	930.6 ^{b}	491.7 ^{a}	35.1 ^{a}	83.3 ^{a}
1.0 M x 3	166.0 ^{a}	1613.0 ^{a}	1142.3 ^{a}	491.7 ^{a}	35.2 ^{a}	83.5 ^{a}

Means within columns without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey‘s test.

Table 4.6 shows that in accordance with the classification table of soil pH, sand is considered as a strongly acid. Furthermore soil pH increased with increased frequency and concentration of NaCl application. Data show that the highest values were associated with three times 1.0 M NaCl irrigation and the lowest with the control. Electrical conductivity (EC) decreased with increased salt concentration in the sand (Table 4.6). Lowest values of soil electrical conductivity were observed with three times 1.0 M NaCl irrigation and EC decreased with increased frequency and concentration of irrigation.

Table 4.6: Effect of NaCl irrigation on pH and EC on medium in greenhouse at the end of the experiment

	Control	0.5 M NaCl			1.0 M NaCl		
		Once	Twice	Three	Once	Twice	Three
pH	4.12	4.22	4.42	4.88	4.55	4.99	5.11
EC (dS m^{-1})	2.37	2.35	2.32	2.29	2.33	2.25	2.12

4.4.1.9 Mineral content of plants in the greenhouse

The results indicate the ion content of the plants under salinity stress and these ions important roles in plants under NaCl stress. The Na^+ concentration of plants increased progressively with increased irrigation with NaCl (Table 4.7). Na^+ levels were significantly higher in plants irrigated with 1.0 M than in the controls and irrigation with 0.5 M. NaCl generally increased Na^+ levels significantly above those with 0.5 M NaCl. The concentration of K^+ decreased significantly with salinity irrigation but did not exhibit any definite relationship with concentration and frequency of NaCl. Mg^{++} decreased significantly while concentration of Ca^{++} and P increased.

Table 4.7: Concentration of potassium, calcium, sodium, magnesium, iron and phosphorus of plants in the greenhouse at the end of the experiment

NaCl treatment	Concentration (mg kg^{-1})					
	K	Ca	Na	Mg	Fe	P
Control	7087.1 a	4958.8 ^{b}	388.47 ^{e}	843.1 ^{a}	370.1 ^{a}	718.8 ^{a}
0.5 M x 1	6646.8 ^{b}	4902.5 ^{b}	9509.4 ^{d}	602.5 ^{b}	357.1 ^{a}	746.9 ^{a}
0.5 M x 2	6854.3 ^{b}	4995.7 ^{b}	14373.5 ^{d}	385.1 ^{b}	318 ^{ab}	846.5 ^{a}
0.5 M x 3	6802.9 ^{b}	6647.1^{a}	16795.9 ^{bd}	366.2 ^{b}	284.1 ^{ab}	870.1 ^{ab}
1.0 M x 1	6504.5 ^{b}	5411.1^{ab}	18192.2 ^{b}	328.8 ^{c}	275.7 ^{b}	872.7 ^{b}
1.0 M x 2	6401.2 ^{b}	6582.6 ^{a}	22204.6 ^{a}	250.7 ^{d}	185.3 ^{c}	951.5 ^{c}
1.0 M x 3	Died	Died	Died	Died	Died	Died

Means within columns without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Ca^{++}/ Na^{+} and K^{+}/Na^{+} ratios in plants decreased with salinity treatment. Increasing salinity level decreased the ratios of K^{+}/Na^{+} and Ca^{++}/Na^{+}, and there were considerable differences between saline treatment and treatment under no salinity. The values of Ca^{++}/Na^{+} and K^{+}/Na^{+} ratios in the seedlings decreased with increased concentration and frequency of NaCl irrigation (Table 4.8).

Table 4.8: Effect of NaCl irrigation on $K^{+/}Na^{+}$ and Ca^{++}/Na^{+} ratios on plants in greenhouse at the end of the experiment

	Control	0.5 M NaCl			1.0 M NaCl		
		Once	Twice	Three	Once	Twice	Three
Ca^{++}/Na^{+}	12.8	0.52	0.36	0.40	0.30	0.28	Died
K^{+}/ Na^{+}	18.2	0.70	0.48	0.41	0.36	0.29	Died

4.4.2 Field experiment in Libya

4.4.2.1 Plant survival

Figure 4.11 shows the decline in percentage survival with time. Emergence was initially more than 75% in all treatments. Survival was significantly reduced with treatment with 0.5 M NaCl twice and three times and 1.0 M NaCl more than once, compared to controls.

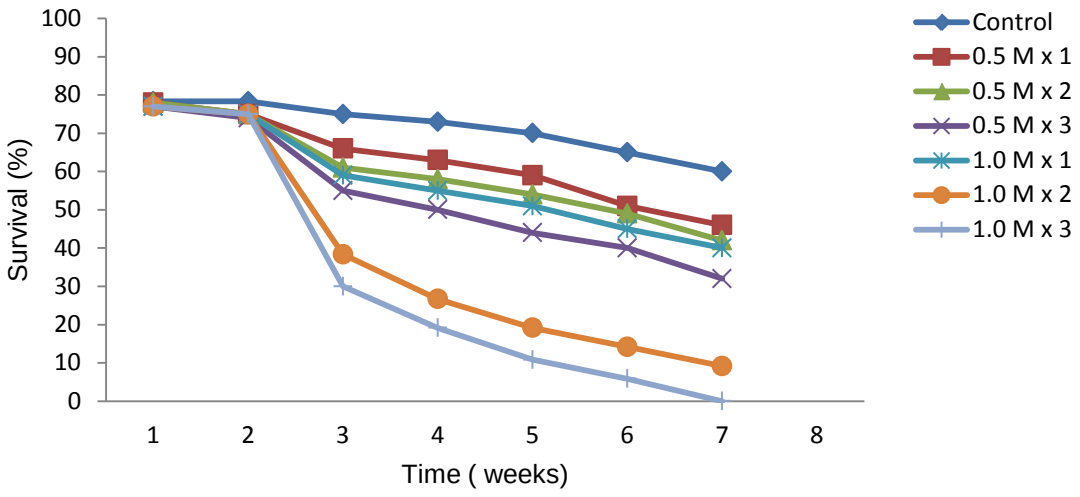

Figure 4.11: Effect of frequency and concentration of NaCl irrigation on survival of *Acacia saligna* in the field in Libya.

4.4.2.2 Shoot fresh and dry weight

Figure 4.12 show that there was a significant effect of saline irrigation on shoot fresh weight which was significantly lower with 1.0 M NaCl than with 0.5 M NaCl. Shoot fresh weight also decreased significantly with increased frequency of irrigation. Again, plants treated with low salt levels had higher fresh weight than those treated with high concentration. All plants treated two or three times with 1.0 M NaCl died.

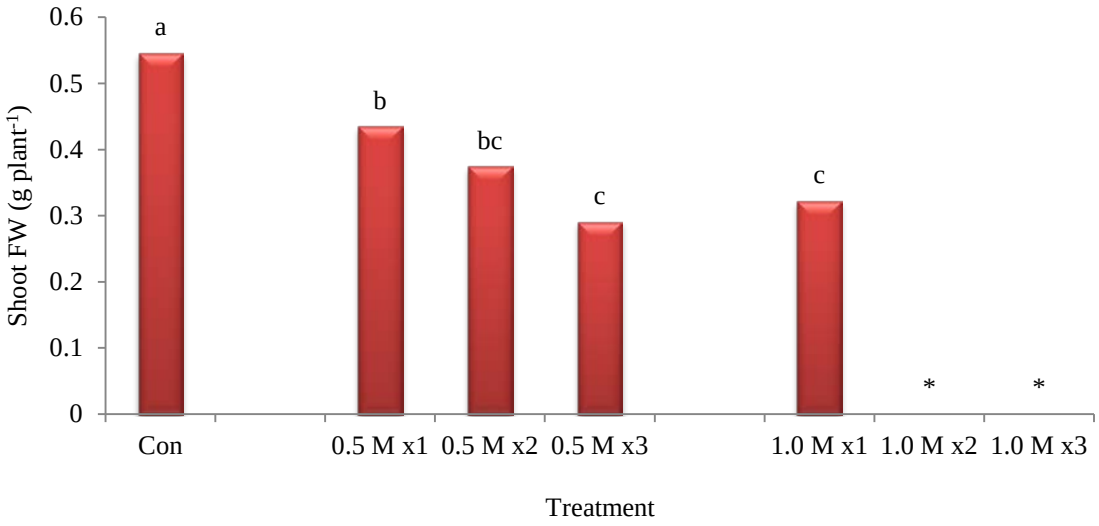

Figure 4.12: Effect of salinity on shoot fresh weight of surviving plants after 7 week in the field in Libya. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Figure 4.13 shows that dry weight of plants treated with saline irrigation was significantly decreased compared with the control. Dry weight was significantly affected by both concentration and frequency of saline irrigation.

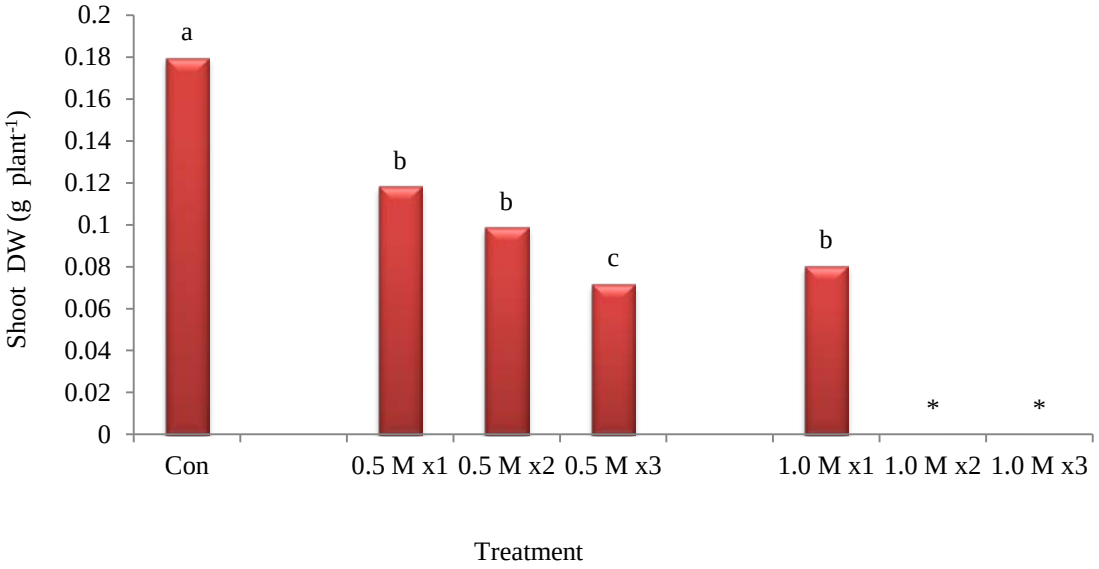

Figure 4.13: Effect of salinity on shoot dry weight of surviving plants after 7 weeks in the field in Libya. Mean without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

4.4.2.3 Shoot moisture content and shoot height

Figure 4.14 shows the shoot moisture content of plants treated with NaCl irrigation was significantly higher with both 0.5 M NaCl and 1.0 M NaCl than with the control.

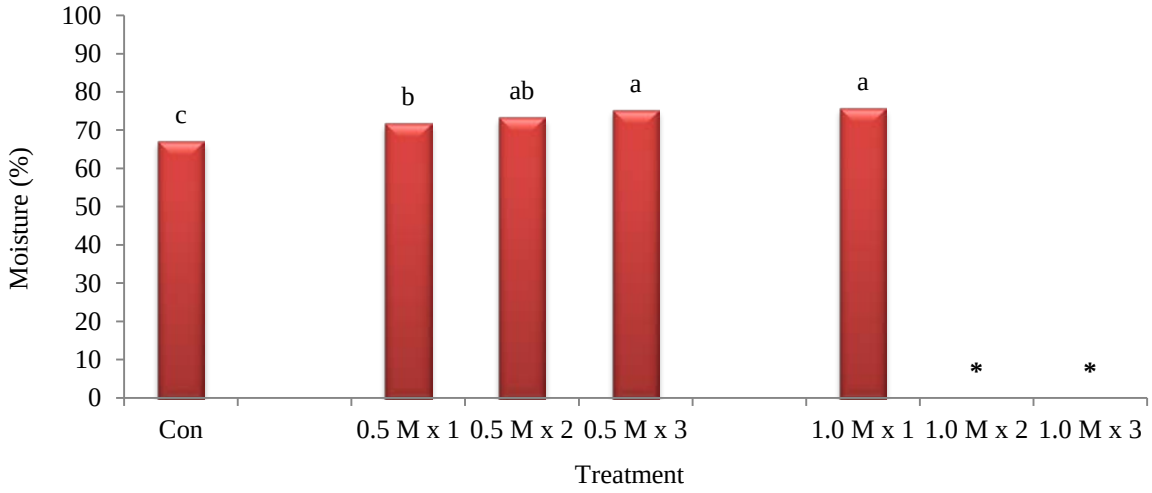

Figure 4.14: Effect of salinity on shoot moisture content of surviving plants after 7 weeks in the field in Libya. Mean without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Figure 4.15 shows that shoot height decreased with increased NaCl concentration and with frequency of irrigation. This was significant for treatment with 0.5 M NaCl twice and 1.0 M NaCl once compared with the controls.

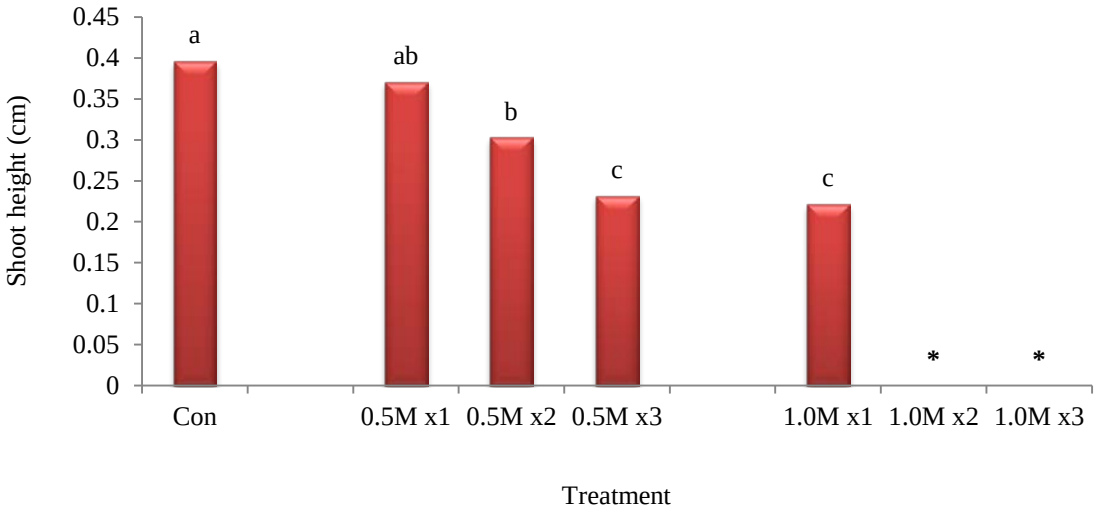

Figure 4.15: Effect of salinity on height of surviving plants after 7 weeks in the field in Libya. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.* All plants died.

4.4.2.4 Root fresh and dry weight

Salinity caused a significant reduction in root fresh and dry weight compared to control plants (Figures 4.16 and 4.17). There was a significant reduction in root fresh biomass caused by one to three applications of 0.5 M NaCl. Root dry weight was significantly reduced by 0.5 M NaCl applied two or three times or 1.0 M NaCl applied once.

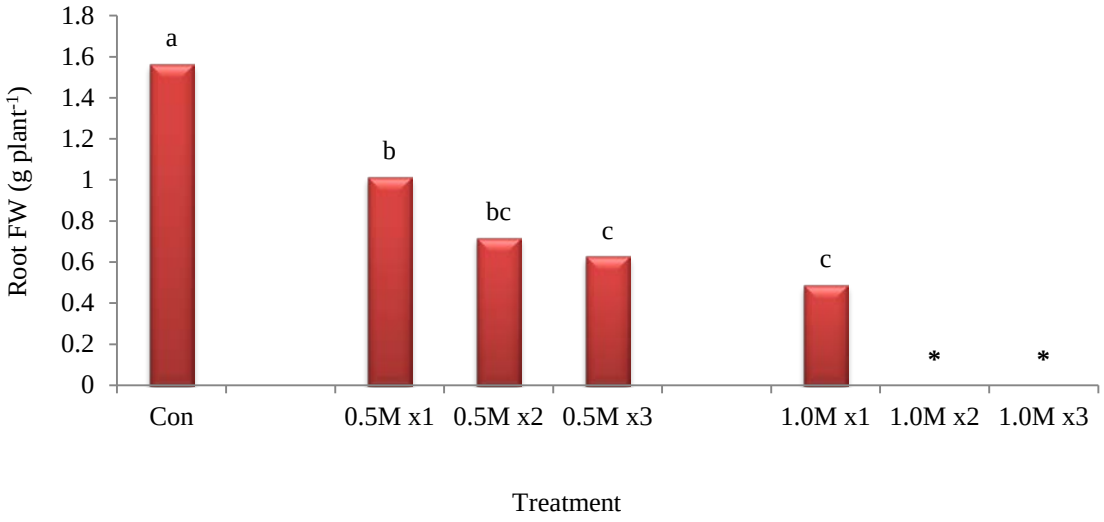

Figure 4.16: Effect of salinity on root fresh weight of surviving plants after 7 weeks in the field. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.* All plants died.

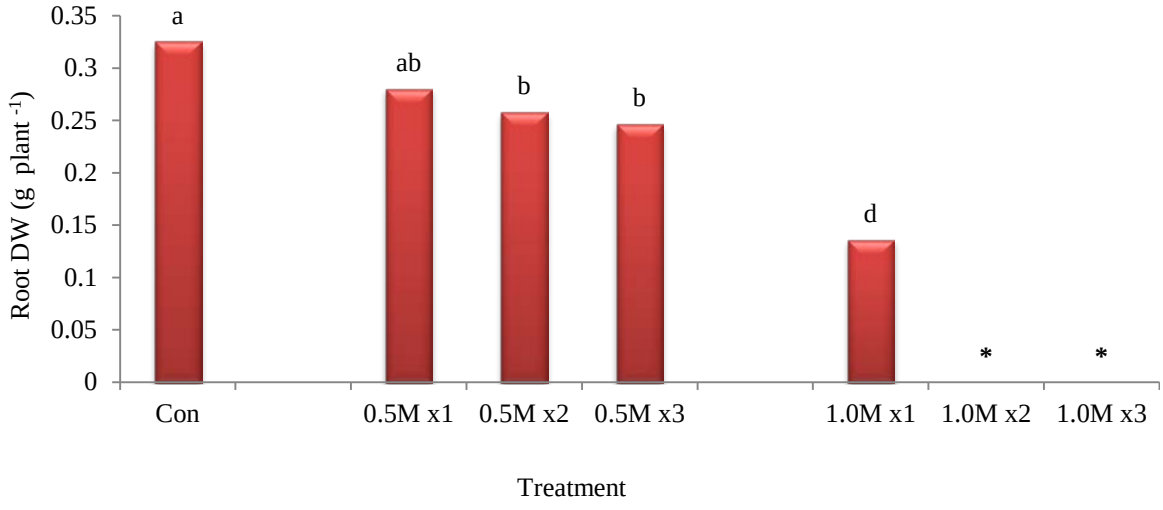

Figure 4.17: Effect of salinity on root dry weight of surviving plants after 7 weeks in the field. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

4.4.2. 5 Root moisture content

Moisture content in roots varied with frequency of NaCl irrigation. It decreased significantly with 0.5 M NaCl applied twice and three times; whilst no significant variation was found between the no salt control and 0.5 or 1.0 M NaCl applied once. Plants showed mortality with two or three times irrigation with 1.0 M NaCl (Figure 4.18). Figure 4.19 shows there were significant reductions in shoot/root ratio when irrigated with 0.5 M twice or three times and 1.0 M NaCl.

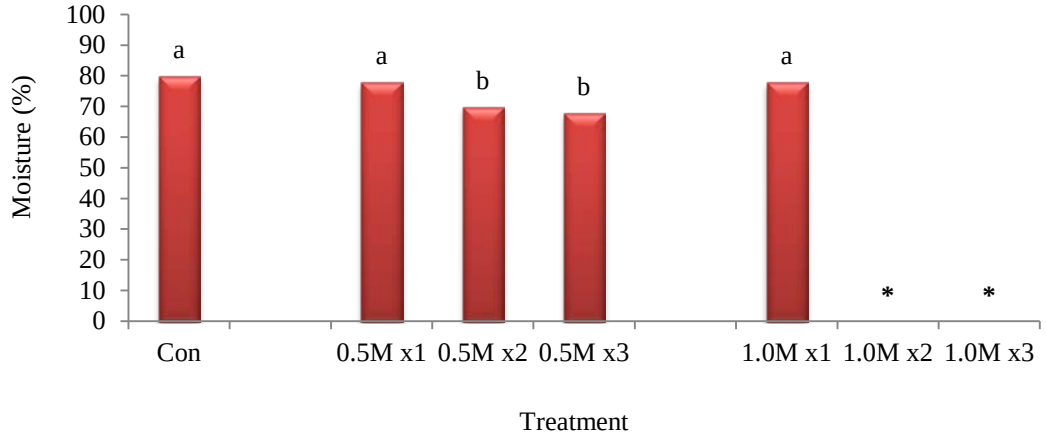

Figure 4.18: Effect of salinity on root moisture content of surviving plants after 7 weeks in the field. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

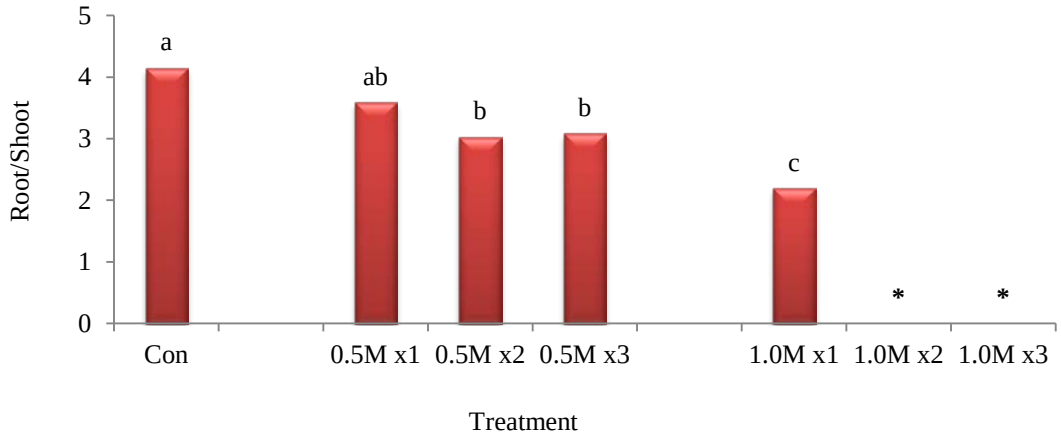

Figure 4.19: Effect of salinity on root /shoot ratio of surviving plants after 7 weeks in the field. Means without a letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

4.4.2.6 Mineral content in the medium in the field in Libya

A summary of the physical and chemical properties of the soil of Sidi El Mesri in the field in Tripoli Libya at the beginning of the experiment are presented in Tables 4.9 and 4.10, respectively.

Table 4.9: Physical characteristics of soil of Sidi El Mesri at the beginning of the experiment (values are means of three replicate measurements)

Depth	0-30 cm
Sand (%)	88
Silt (%)	9.6
Clay (%)	2.4
Texture	Sandy loam

Table 4.10: Chemical properties of soil of Sidi El Mesri at the beginning of the experiment (values are means of three replicate measurements)

pH	7.7-7.8
Ec (dS m^{-1})	5.3-5.5
OM (%)	0.08
Total nitrogen (%)	0.007
Elements	Concentration (mg kg^{-1})
K	131.0
Ca	1029.7
Na	523.0
Mg	202.1
Fe	509.3
P	10.02

Table 4.11 shows how increasing irrigation frequency and concentration of NaCl application in the medium significantly increased concentration of Na^+. NaCl treatment also resulted in significantly increased K^+, Ca^{++} Mg^{++} and P concentrations in the medium compared with controls. However, with K^+ there was no significant increase with application of 1.0 M NaCl at any frequency compared to 0.5 M NaCl applied three times, and for Mg^{++}, P and Ca^{++} there was no further increased with three fold irrigation with 1.0 M NaCl compared with two fold. For Fe^{++} significantly increases above control concentration were only found with irrigation with 1.0 M NaCl two or three times.

Table 4.11: Concentration of potassium, calcium, sodium, magnesium, iron and phosphorus of the medium in the field in Libya at the end of experiment

NaCl Treatment	Concentration (mg kg^{-1})					
	K	Ca	Na	Mg	Fe	P
Control	123.7 ^{d}	995.4 ^{e}	531 ^{d}	200.0 ^{c}	469.7 ^{c}	10.3 ^{d}
0.5 M x 1	144.2 ^{c}	1031.7 ^{d}	830 ^{c}	204.5 ^{c}	469.8 ^{c}	13.1 ^{c}
0.5 M x 2	153.2 ^{b}	1043.9 ^{c}	1099 ^{c}	206.6 ^{b}	470.6 ^{c}	13.5 ^{c}
0.5 M x 3	176.3 ^{a}	1056.9 ^{c}	1161 ^{c}	207.7 ^{b}	470.8 ^{c}	15.7 ^{b}
1.0 M x 1	177.8 ^{a}	1064.2 ^{b}	1091 ^{c}	208.0 ^{b}	471.3 ^{c}	16.2 ^{b}
1.0 M x 2	178.9 ^{a}	1071.9 ^{a}	2154 ^{b}	208.5 ^{a}	478.5 ^{b}	18.0 ^{a}
1.0 M x 3	179.8 ^{a}	1071.6 ^{a}	2668 ^{a}	209.6 ^{a}	482.5 ^{a}	18.3^{a}

Means for each column, the value of the same postscript letter are not differ significantly at $p \leq 0.05$ based on two-way analysis of variance an using Tukey‘s test.

Table 4.12 shows that in accordance with the classification table of soil pH, the soil at the experimental site was slightly alkaline. Irrigation three times with 1.0 M NaCl substantially increased pH and EC was decreased with all levels of NaCl application, pH being highest after three applications of 1.0 M NaCl. The lowest values of soil electrical conductivity were observed with three times 1.0 M NaCl irrigation and EC decreased with increased frequency and concentration of irrigation.

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Table 4.12: pH and EC of the medium in the field in Libya at the end of experiment

	Control	0.5 M NaCl			1.0 M NaCl		
		Once	Twice	Three	Once	Twice	Three
pH	7.8	7.10	7.18	7.25	7.20	7.81	8.98
EC (dS m^{-1})	5.51	4.88	4.77	4.42	4.85	4.52	4.45

4.4.2.7 Mineral content of plants in the field in Libya

Table 4.13 shows that the Na^+ and Fe^{++} content of the plants increased progressively with increasing 0.5 M NaCl irrigation compared with controls, while the concentration of K^+, Ca^{++}, Mg^{++} and P decreased with increasing NaCl. The K^+/ Na^+ and Ca^{++}/ Na^+ ratios in the seedlings decreased with the increasing of NaCl application and with increasing frequency of 0.5 M NaCl irrigation (Table 4.14).

Table 4.13: Concentration of potassium, calcium, sodium, magnesium, iron and phosphorus in the plants of *Acacia saligna* in the field in Libya at the end of the experiment

NaCl	Concentration (mg kg^{-1})					
Treatment	K	Ca	Na	Mg	Fe	P
Control	9874.1 ^{a}	13685 ^{a}	186.4 ^{e}	1519.7 ^{a}	147.4 ^{d}	1068.2 ^{a}
0.5 M x 1	9397.8 ^{a}	11438 ^{b}	4353.4^{d}	1103.4 ^{b}	189.4 ^{c}	918.7 ^{b}
0.5 M x 2	7768.3 ^{b}	8123 ^{c}	8140.1 ^{c}	875.6 ^{b}	267.3 ^{b}	833.8 ^{b}
0.5 M x 3	5564.3 ^{c}	5637 ^{d}	12800.7 ^{a}	647.7 ^{d}	329.9 ^{a}	481.1 ^{c}
1.0 M x 1	6343.5 ^{bc}	7567 ^{c}	9848.6 ^{b}	791.9 ^{bd}	241.3 ^{b}	245.1 ^{d}
1.0 M x 2	Died	Died	Died	Died	Died	Died
1.0 M x 3	Died	Died	Died	Died	Died	Died

Means for each column, the value of the same postscript letter are not differ significantly at $p \leq 0.05$ based on two-way analysis of variance an using Tukey‘s test.

Table 4.14: Effect of NaCl irrigation on $K^{+/}Na^+$ and Ca^{++}/ Na^+ ratios of plants in the field in Libya at the end of the experiment

	Control	0.5 M NaCl			1.0 M NaCl		
		Once	Twice	Three	Once	Twice	Three
Ca^{++}/Na^+	73.4	2.6	0.99	0.44	0.77	Died	Died
K^+/ Na^+	53.3	2.2	0.95	0.43	0.64	Died	Died

4.5 Discussion

Salinity stress of a plant is a combination of water stress (osmotic) and ion imbalance toxicity stress. Salt stress arises from excessive uptake of salts by plants and is a specific and unavoidable consequence of high ion concentration. Ion imbalance especially results from altered ionic ratio in the cells after accumulation of the dominant (mostly Na^+ and Cl^-) ions of the salt which is responsible for the salinity of the medium.

Survival in the greenhouse and field in the experiments described was significantly reduced by salinity. Salinity treatment significantly reduced plant survival (Figures 4.1 and 4.11) with concentration and frequency of NaCl irrigation both having a significant influence on plant survival. Survival was not affected with one 0.5 M NaCl irrigation but increased frequency of irrigation reduced plant survival with three irrigations of 0.5 M NaCl and only one of 1.0 M NaCl. However, with three times irrigation with 1.0 M NaCl all plants died in the greenhouse and field.

Increasing NaCl concentration reduced growth of *A. saligna* in the field when irrigated twice with 1.0 M NaCl. On the other hand there was some growth at lower NaCl concentrations in the greenhouse but not in the field when irrigation with 0.5 M NaCl once. This result agree with Ansari, Khazada, and Azmi (1988) who reported a stimulation of growth with 1.0 M and 1.5 M NaCl irrigation in pot trials with 6 month old seedlings of *Acacia stenophylla, A. ampliceps, A. auriculiformis, A. maconchieana* and *A. bivenosa*. *A. saligna* establishment does not depend only on the concentration of NaCl but also on frequency of irrigation. The plant survival with more than one irrigation decreased with increasing NaCl concentration. Aswathappa, Marcar, and Thomson (1987) compared the

salinity tolerance of thirty seven species of *Acacia* in an experiment with 2 month-old seedlings treated in stepwise increments of 25 mM every 2 days. They found irrigation with NaCl affects plant growth due to osmotic stress and toxicity of Na^+ and Cl^- ions and nutrient imbalances. Several investigations have reported plant growth reduction as a result of salinity stress, e.g. in tomato (Romero-Aranda, Soria, and Cuartero 2001), cotton (Meloni *et al*. 2001) and sugar beet (Ghoulam and Fares 2001). There were large interspecific differences although there was no consistent pattern in the response of different parameters to NaCl.

The effect of salinity on some growth and yield parameters of *A. saligna* included a decrease in fresh weight with increasing NaCl concentration after seven weeks. Significant differences in fresh weight were obtained between the plants treated with the lower NaCl (twice and three times irrigation with 0.5 M NaCl) and those with the higher NaCl (once, twice and three times irrigation with 1.0 M NaCl) as shown in the Figures 4.2 and 4.12. An increase in the salinity of irrigation water from 0.5 M to 1.0 M NaCl resulted in marked decreased in the fresh weight of the plants.

The dry weight of plants irrigated with 0.5 M NaCl differed significantly when compared with dry weight treated with higher 1.0 M NaCl such that the higher concentration of NaCl, the lower the dry weight. This decrease in growth probably results from the deceased availability of water and increased toxicity of NaCl in the root media, produced by increased salinity of irrigation water.

The mean values of shoot fresh and dry weight were reduced at the end of experiment. This result agrees with El-Lakany and Luard (1986) who also worked with *Acacia* spp. in a greenhouse, increasing salinity by 100 mM every 3 weeks from 50 to 650 mM NaCl.

This reduced growth of most *Acacia* spp., although lower concentrations produced some growth stimulation in the most tolerant *Acacia*s. In their studies the parameters that were significantly increased by low NaCl concentration were plant survival, shoot fresh and dry weight compared with high concentrations. Frequency of saline water irrigation affects biomass of plants. Increasing frequency of irrigation with saline water with either 0.5 M or 1.0 M NaCl decreased plant growth substantially. From treatments receiving three times irrigation with 1.0 M NaCl where all plant died. Where plants survived, performance was poorest with 1.0 M irrigation applied once or twice. Concentration and frequency of irrigation whether with 0.5 M or 1.0 M NaCl influenced root fresh and dry weight of plants. However, when 0.5 M level was used it produced more root fresh and dry weight than 1.0 M NaCl in the greenhouse and in the field in Libya (Figures 4.5, 4.6, 4.16 and 4.17).

It seems from the results that irrigation with 1.0 M NaCl once, twice and three times would be harmful to *A. saligna* growing in a greenhouse. The severity of the salinity effect was greater in the field in Tripoli, Libya than in the greenhouse, which is a response mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al*.1994). These results agree with Aziz and Khan (2001) who found that the optimum growth of *Rhizophora mucronata* plants was obtained at 50% seawater and declined with further increase in salinity, while in *Alhogi pseudoalhagi* (a leguminous plant), total plant weight was increased at low salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl), but leaf number was less affected. Neumann (1997) considered that inhibition of leaf growth by salt decreases the volume of new leaf tissues into which excess salt can be accumulated and, combined with continuous salt accumulation; it could lead to earlier build up of excess salt levels. The results of larger decrease in accumulation of dry matter in shoots than in roots,

particularly at high NaCl concentration, agrees with Ghoulam and Fares (2001), working with sultana vines who recorded a larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, indicating partitioning of photoassimilates in favour of roots. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by the roots.

Plant height decreased markedly with increased concentration and amount of NaCl. Salinity decreased plant height with increasing NaCl irrigation. Irrigation with 0.5 M NaCl once did not have any significant effect on plant height, but plant height was reduced with 0.5 M NaCl twice and three times and with 1.0 M NaCl irrigation. The reduction in plant height was more pronounced with 1.0 M NaCl irrigation especially with three times when compared with 0.5 M NaCl or no NaCl (control). The effect of salt stress on the number of leaves was similar to that on plant height.

Moisture content in shoots and roots also varied with salinity treatment. No significant affect was found between irrigation with 0.5 M NaCl once and the control. High salinity caused a reduction in the moisture content in shoot and root in experiments in the greenhouse but not in the field (Figure 4.4, 4.7, 4.14 and 4.18). Glenn (1987) reported that the water content of 19 grasses declined with an increase in salinity. Water content in *A. saligna* decreased with frequency of saline irrigation being at its lowest after three applications of salt. This decrease in water content by salinity could be attributed to low ion accumulation in the shoot tissue and to osmotic balance by reducing the tissue water (Gulzar, Ajmal Khan, and Ungar 2005).

Salinity can affect plant growth by reducing the amount of water available and by increasing the concentration of certain ions that have a toxic effect on plant

metabolism (Wahome, Jesch, and Grittner 2001). Salinity affects plant physiology through changes of water and ionic status in the cells According to Dubey (1997) and Yeo (1998) salt causes both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects. The general response of plants to salinity is a reduction in growth (Romero-Aranda, Soria, and Cuartero 2001, Ghoulam and Fares 2001). The initial and primary effect of salinity, especially at low to moderate concentration, is due to its osmotic effects (Munns and Ternaat 1986, Jacoby 1994). Osmotic effects of salts on plants are the results of lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil potentials, this condition interferes with the plant's ability to extract water from the soil and maintain turgor.

At high salinity, some specific symptoms of plant damage may be recognized such as necrosis and leaf tip burn due to Na^+ or Cl^- ions. High ionic concentration may disturb membrane integrity and function; interfere with internal solute balance and nutrient uptake, causing nutritional deficiency symptoms similar to those that occur in the absence of salinity (Grattan and Grieve 1999). Salt stress arises from excessive uptake of salts by plants and is a specific and unavoidable consequence of high ion concentration. Ion imbalance specially results from altered ionic ratio in the cells after accumulation of the dominant (mostly Na^+ and Cl^-) of the salt which is responsible for the salinity of the medium. Irrigation with different concentrations of NaCl results a significant accumulation of Na^+ and decrease in K^+, Ca^{++}, Mg^{++}, P and Fe^+ ions.

pH of soil irrigated with 0.5 M NaCl or 1.0 M NaCl was higher than in the control. It was 7.10 in the control and increased to 7.25 with 0.5 M NaCl and 8.10 with 1.0 M

NaCl. This could be attributed to the displacement of H^+ ions from the soil, caused by the addition of bases. *A. saligna* production can be decreased as a result of high levels of soil pH as well as by the reduction of water movement into the medium or soil (Allen and Johnson 2007). In the experiment in the field in Libya, pH was higher than 8.5 and the soils are considered to be alkaline soils and this causes some essential nutrients such as magnesium (Mg) and calcium (Ca) to be unavailable (Soil Quality Information Sheet 1998). Conversely, if the soil pH declines below a critical level, the solubility of aluminium and manganese ions increases, resulting in toxicity and lower yields. Soil acidity affects plant growth in several ways. Toxicity, caused by increased mobility of soil aluminium, is thought to be the most serious of these effects (Black 1992). Aluminium becomes available when the pH drops below about 5.5. The cation exchange complex of soils becomes largely saturated with aluminium ions at pH 4.0. As a result, plants are deprived of essential cations (Foth and Ellis 1997). In the greenhouse experiment a soil pH below 5.5 was found adversely so that this affects roots.

Saline water resulted in highly significant decrease in the electrical conductivity (EC) of irrigated soil compared with that of soil irrigated with just distilled water in the greenhouse (Table 4.6) and in the field in Libya (Table 4.12). Chemical and physical characteristics of plants are affected by high concentrations of salinity in the soil and by high concentrations of exchangeable sodium in the soil. Electrical conductivity (EC) was decrease with the increase of the salt concentration in the medium (Table 4.6, and 4.12). The results of greenhouse and field experiment for *A. saligna* irrigation with saline water, one time irrigation with 0.5 M NaCl at the establishment stage may be better than two or three times irrigation and only one with 1.0 M NaCl.

The effect of salinity is determined by the effect on growth, as well as by the ionic concentration in the cell. The high ionic concentration in the cell is responsible for most of the biochemical changes that affect the cellular metabolic level, and consequently the growth and development of the plant. High salt (NaCl) uptake competes with the uptake of other nutrient ions, such as K^+, Ca^{++}, N and P resulting in nutritional disorders and eventually, reduced yield and quality (Grattan and Grieve 1999). Increased NaCl concentration has been reported to induce an increase in Na^+ and decreases in Ca^{++}, K^+ and Mg^{++} levels in a number of plants (Khan, Ungar, and Showalter 2000, Bayuelo-Jimenez, Debouck, and Lynch 2003).

The plants studied in both the greenhouse and the field in Tripoli, Libya showed a significant increase in the amount of Na^+ in plants with increase in salinity of the external medium. Plants may respond to salinity by absorbing sodium at high rates and accumulating these ions in their leaves for osmotic adjustments to the low water potential in the soil. There was a relationship between increased Na^+ content in plants and increased salt concentration in soil. The increase in Na^+ content of *A. saligna* was due to the high Na^+ content of irrigation water (Tables 4.5, 4.7 and 4.11, 4.13). This result agrees with Ghoulam and Fares (2001) who observed an increase in Na^+ and Cl^- content in the leaves and roots of sugar beet with increasing NaCl concentration in the rooting medium.

The significant decrease of K^+ in plants with an increase in irrigation water salinity might be due to the competition phenomenon between Na^+ and K^+ because Na^+ content was higher than K^+ in the irrigation water with NaCl. This results in absorption of more Na^+ than K^+ by the plants. This result contradicts Parida, Das, and Mittra (2004) who

reported no alteration of the endogenous level of K^+ in leaves with a significant increase in Na^+ content in leaves, stem and root of the mangrove (*B. parviflora*). Calcium concentration decreased significantly with 0.5 M and 1.0 M NaCl irrigation compared with the control plants in the field but increased in the greenhouse and this may be due to higher temperature and higher rate of evaporation which would cause rises in salt concentration in the soil and the growth and survival of *A. saligna* were markedly reduced.

Mg^{++} also showed a significant decrease in plants in the field and in the greenhouse with increased salt concentration of the irrigation medium. Salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by plants as reviewed by Villora *et al*. (1997) and Grattan and Grieve (1999). Decreases in Mg^{++} content of the leaves have been reported upon salt accumulation in the plant. All salinity treatments caused a significant reduction in Mg^{++}. These results agree with Grattan and Grieve (1999) who found a reduction in nutrient ions such as K^+, Ca^{++}, N and P resulting in nutritional disorders and, eventually, reduced yield and quality of wheat. Also Perez-Alfocea, *et al*. (1996), Khan, Ungar, and Showalter (2000) and Bayuelo-Jimenez, Debouk, and Lynch (2003) reported that increased NaCl concentration caused on increase in Na^+ and Cl^- and decreases in Ca^{++}, K^+ and Mg^{++} levels in a number of plants.

Salinity affected the phosphorus concentration in the plant which was significantly decreased in plants ($p < 0.001$) in the field but significantly increased in plants in the greenhouse. An overall change in P concentration in plants with increasing salt concentration is in agreement with Ansari (1990) who indicated that salinity either

increased or had no effect on P uptake. In most cases, salinity decreases the concentration of P in plant tissue Sharpley, Meisinger, and Suarez (1992) but the results of some studies indicate salinity either increased or had no effect on P uptake. Awad, Edwards, and Campbell (1990) in a study on tomato plants in a greenhouse using flow-through solution cultures maintained low levels of phosphate and a beneficial response was observed as phosphate concentrations increased from 0.1 to 10 mM. In addition, absence or low concentration of other cations (K^+, Ca^{++} and Mg^{++}) intensified the movement of Na^+ in the shoot with the concentration increasing with the level of exchanged Na^+.

The concentrations of K^+, Ca^{++} and Mg^{++} in the leaves of most studied species are a little higher in the presence of the low salt concentration, but at higher salt concentrations they are reduced. A. *saligna* growth is adversely affected by saline water irrigation due to one or more of the following factors: firstly, high exchangeable sodium percentage in saline water influences markedly the physical soil properties. As exchangeable sodium percentage increases, so the soil tends to become more dispersed which results in the breakdown of soil aggregates and this lowers the permeability of the soil to air and water. Dispersion also leads to the formation of dense, impermeable surface crusts that hinder the emergence of seedlings and concentration of Na^+ affect on the concentration of K^+, Ca^{++} Mg^{++} Fe and P in the leaves of plants were little better in the presence of the low salt concentration.

The high salt concentration in the nutrient medium leads to an increase in ion uptake. This lowers the water potential in the plant roots and increased water uptake.

4.6 Conclusion

A study of the salt tolerance of *A. saligna* is important to obtain information that can be used to help this species to grow effectively in saline habitats or when saline water is used for irrigation.

Increasing NaCl concentration progressively reduced plant survival of *A. saligna*. With low concentration (0.5 M NaCl) it was able to survive repeated application of saline irrigation water, without significant harm in both experiments. NaCl irrigation resulted in all plants irrigated more than once or twice with 1.0 M being shorter with fewer leaves.

Increasing NaCl concentration and frequency progressively reduced shoot fresh and dry weight. Fresh and dry weight of shoots and roots began to decrease with 0.5 M NaCl applied more than once.

Increasing NaCl content in the media led to increasing Na^+ concentration in plants which induced high differences in nutrient contents via changes in absorption and reduced uptake of other mineral nutrients such K^+, Ca^{++} , Mg^{++} and P ions and decreased K^+/Na^+ and Ca^{++}/Na^+ ratio under saline condition.

It could be concluded that *A. saligna* can be classified as a moderately salt tolerant plant. If irrigation with saline water is necessary, one time irrigation with 0.5 M NaCl at the establishment stage may be better than two or three times irrigation and only one with 1.0 M NaCl after once concentration all plants died in the field experiment.

Chapter 5

Effect of Zander on the growth of *Acacia saligna* under saline conditions

5.1 Introduction

Arable land resources are under increasing pressure as the world population increases especially in the arid and semi-arid regions. The effect of population pressure on resources is increasing rapidly and, as a consequence, the demand for trees or wood has increased even faster than the population. This has caused desertification and raised concerns about a shortage of tree production and environmental degradation. Salinity is one of the world's oldest and most serious environmental problems. It has been estimated that mismanagement of irrigation projects has resulted in salinity which is reducing yields. Some of the most serious examples occur in the semi-arid regions; however, large areas of saline soils also occur in humid regions, particularly in coastal areas. The best strategy to overcome the above problems may be to grow woody vegetation that can be economical and beneficial to local communities and also protect the environment from increased saline irrigation. Perennial legume trees and shrubs have attracted considerable interest as multipurpose species in a wide range of climatic zone (Harris 1988, Harris *et al*. 1989). In Libyan Jamahiriya the climate consists of 5-6 months of hot, dry summer with a short, warm, wet winter. Much of the annual rainfall occurs over the winter months of October to March with average rainfall of 350 mm in Tripoli Province where summer temperature ranges from 35 to 45°C and winter temperature from 5 to 27 °C. Due to limited good quality water resources in Libyan Jamahiriya and overexploitation, there are shortages of high quality ground water for agriculture, industrial and domestic use. Such overexploitation has caused the deterioration of the water quantitively and qualitatively. This has led to thinking about

the use of such saline water for irrigation, irrigation water management, type of crops to be irrigated, soil and water salinity monitoring, leaching of salts, proper irrigation systems to fit such water and addition of organic amendments to decrease the effect of salinity on plant growth and yield. This will save the good quality water for domestic use, and irrigation of sensitive crops and industry. Soil amendment can include virtually any substance that improves the growth of plants in soil. There are two broad categories of soil amendments: organic and inorganic. Organic amendments come from something that is or was alive. Inorganic amendments, on the other hand, are either mined or man-made. Organic amendments include sphagnum peat, wood chips, grass clippings, straw, compost, manure, bio-solids, sawdust, wood ash and Zander. Inorganic amendments include vermiculite, perlite, pea gravel, sand, lime and pumice. Generally, fertilizers differ from soil amendments by their higher nutrient content, but the distinction is not always clear. Soil amendment can improve both chemical and physical properties of a soil; the chemical properties that may be altered by soil amendment include the soil fertility and pH. Organic amendments increase soil organic matter content and offer many benefits. Organic matter improves soil aeration, water infiltration, and both water- and nutrient-holding capacity. Many organic amendments contain plant nutrients and act as organic fertilizers. Organic matter also is an important energy source for bacteria, fungi and earthworms that live in the soil. The physical properties of soil that are improved by amendment include soil structure. Soil organic matter increases the amount of water the soil can hold and the proportion of water available for plant growth. In addition, it is a major source of the plant nutrients phosphorus and sulphur, and the primary source of nitrogen for the plants. Soil organic matter greatly influences the biology of the soil, because it provides most of the food for the community of heterotrophic soil organisms. Brady and Weil (1999) suggested that

there are direct and indirect factors contributing to the favourable effects of organic matter on soil water availability. Genon and Dufey (1991) suggest that the cation exchange capacity (CEC) is the most important soil chemical property with respect to mineral nutrient retention and bioavailability. Zander is a fibre amended mineral material, originating in lacustrine sediments derived from naturally occurring organic deposits laid down over several thousand years. A sustainable system of anaerobic decomposition is associated with fresh water systems in many parts of the world. It is therefore relatively small amounts can have a lasting effect in areas of the world affected by overgrazing, over-cultivation and desert encroachment (Zander Corporation 2007). This chapter describes the analysis of Zander physical and chemical properties. The overall objective of this chapter was to estimate the effect of saline irrigation on survival fresh and dry weight of *A. saligna* grown in medium amended with different Zander levels.

5.2 Materials and methods

Two experiments were carried out, one in the field and one in the greenhouse. The field experiment was conducted in the field of the Faculty of Agricultural Experiment Farm in Sidi El Mesri Area in Tripoli Libya from July to September 2007. Environmental data are given in Appendix 1. The area which was selected for the experiment is one of the most important irrigated agriculture areas in Libya. It represents 75% of the irrigated area in Libya. The soils are mainly sandy soils with deep soil profile. The greenhouse experiment was carried out at Coventry University, England from March to June 2007. The temperature in the greenhouse during the entire growth period was 25-27^{o}C and the average relative humidity ranged from 40 to 60%. Seeds of *A. saligna* were obtained from Setropa BV, Troelstralaen 4, 1272 JZ Huizen, The Netherlands and Zander was

supplied by the Zander Corporation, UK. The same design was used for the field and greenhouse experiment except that in the greenhouse Zander was mixed with sand while in the field it was mixed with the soil. Preliminary experiment used growing medium with Zander added to soil at six different concentrations (0, 5, 10, 15, 20 and 30% by volume). *Acacia saligna* responded positively to Zander amendment especially at the higher concentrations. Zander treatment at 30% produced taller plants and more plant biomass than 15 or 20% Zander. They survived, grew well and produced good yields of leaves. To avoid severe growth reductions the system appears to be practicable and needs a relatively high concentration of Zander.

Therefore, three different levels of Zander (0%, 10% and 30%) were applied in this study. Three concentrations of salt irrigation (0.0, 0.5 M and 1.0 M NaCl) were also investigated in this study. Seven treatments were applied; 0.5 M NaCl x 1 (once), 0.5 M x 2 (twice), 0.5 M x 3 (three times) and 1.0 M x 1 (once), 1.0 M x 2 (twice), 1.0 M x 3 (three times) and a no salt (water only) control. The design employed in this experiment was a Randomized Block Design (RBD) with five replicates of each treatment. One hundred and five plastic pots (13 cm diameter) were filled with sand in the greenhouse and soil in the field mixed with Zander. Pots with no Zander were filled only with sand or soil. Sand or soil and Zander were mixed together by weight at 10% Zander + 90% sand or soil and 30% Zander + 70% sand or soil. All pots were irrigated with distilled water to field capacity and any leachate was retained in the saucers. By the third week, all the plants, except the control plants, were watered with 50 ml of 0.5 M or 1.0 M NaCl solution. The fourth week, only the plants with two applications of salt were watered with an additional 50 ml more of 0.5 M or 1.0 M NaCl and the rest were watered with distilled water and the fifth week, plants with three applications of salt

were watered again with 50 ml of 0.5 M or 1.0 M NaCl solution and the rest with distilled water. To avoid plant death plants were watered with distilled water at the last week before harvesting (Table 5.1).

Table 5.1 Irrigation applied on each occasion to pots (DW= Distilled water ml)

Time	Control	0.5M NaCl			1.0 M NaCl		
		0.5 M x1	0.5 M x 2	0.5 M x3	1.0M x1	1.0 M x2	1.0 M x3
WK1	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.
WK2	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.
WK3	D.W.	50 ml	50ml	50ml	50 ml	50 ml	50 ml
WK4	D.W.	D.W.	50 ml	50 ml	D.W.	50 ml	50 ml
WK5	D.W.	D.W.	D.W.	50 ml	D.W.	D.W.	50 ml
WK6	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.	D.W.
WK7	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest

5.2.1 Sample preparation and analysis

5.2.1.1 Soil and Zander analysis

Zander alone and soil with Zander mixes contained in each pot were air dried. After being milled with a 2 mm mesh sieve, each treatment of soil was put in a small bag and labelled for subsequent analysis. The methods used in Chapter 5 to analyse sand and soils was also used for analysis of Zander in the laboratory, including pH, water holding capacity, EC and major ions uptake.

5.2.1.2 Plant growth parameters

All plants from each pot were harvested in mid May 2007 in the greenhouse and mid September 2007 in the field in Libya. The plants were harvested carefully by removing

by hand from the pots and washing the roots free of sand in the greenhouse and soil in the field in Libya with running water. Plants were carefully blotted dry with tissue and fresh weight was determined immediately. Plants were separated into root and shoot using scissors; plant height was recorded and number of leaves was counted. Plants removed were put into plastic bags after harvesting to keep them fresh until they could be taken to the laboratory. Plants were removed from plastic bags and placed in an air circulation oven at 80°C for 24 h, to dry then allowed to cool to room temperature in a desiccator and reweighed again for dry weight of roots and shoots. The methods used in Chapter 5 to analyse plants were also used for analysis of plants from the greenhouse and from the field in Libya.

5.2.2 Statistical analysis

The significance of differences between means was tested by two-way analysis of variance using Minitab Computer Package 15. The mean separation was achieved by the calculation of a least significant difference for all pairs comparisons using Tukey's test at $p \leq 0.05$. Final survival data was arcsin transformed before analysis.

5.3 Results

5.3.1 Greenhouse experiment

5.3.1.1 Seedling survival

Figure 5.1 shows that survival of plants grown in sand alone (0% Zander) decreased with exposure to saline irrigation two or three times with 0.5 M NaCl and once or more with 1.0 M NaCl. Survival was lower with increasing concentration of NaCl and with frequency of irrigation with NaCl. Addition of Zander at 10% to the sand significantly

increased survival of plants grown without NaCl stress, and this was further significantly increased with 30% Zander. With all frequencies and concentrations of NaCl stress applied, survival of plants was significantly greater with 10% Zander than with sand alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander. Survival of plants grown with Zander 30% was unaffected by 0.5 M NaCl compared with unstressed plants in the same medium. With the highest stress applied, 1.0 M NaCl three times, survival was approximately 35% with 10% Zander and 46% with 30% Zander, whereas no plants survived in sand alone (0% Zander). When plants were grown in 10% Zander survival when irrigated with 0.5 M NaCl once or twice was greater than with unstressed plants in 0% Zander, and this was the case with 30% Zander and irrigation with 0.5 M NaCl one, two or three times, and with 1.0 M NaCl once, indicating the beneficial effect of Zander on plant survival under saline stress.

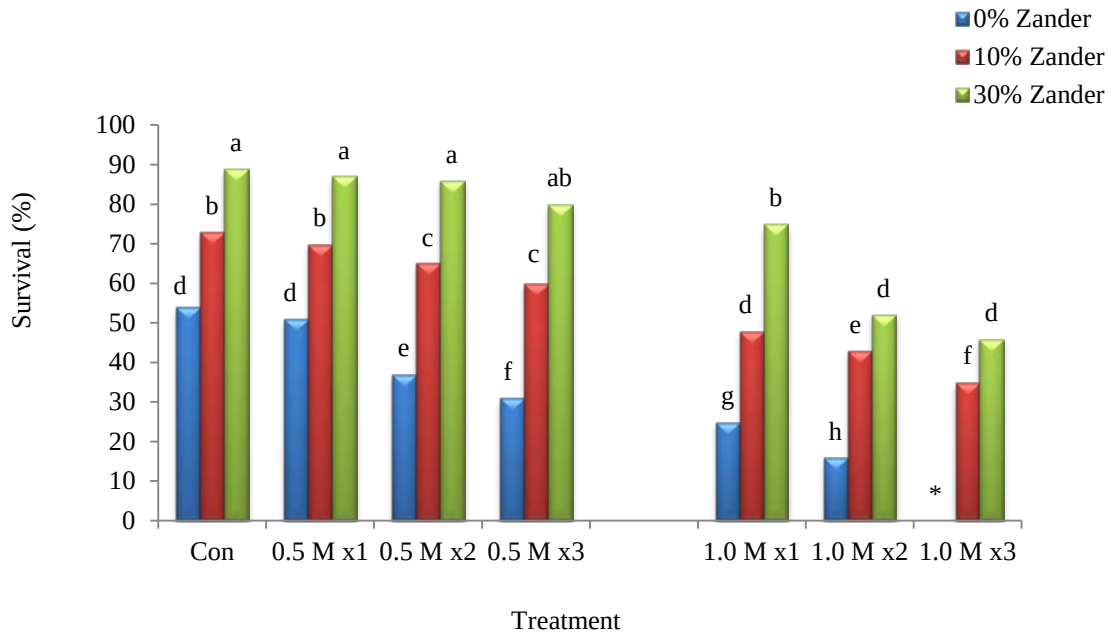


Figure 5.1: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on the survival of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

5.3.1.2 Number of leaves

Figure 5.2 shows that irrigation with 0.5 M NaCl more than once or 1.0 M NaCl significantly decreased the number of leaves on surviving plants in sand alone compared with unstressed plants. Number of leaves decreased with increasing concentration and frequency of NaCl irrigation. In the absence of NaCl stress plants grown with 10% Zander produced significantly more leaves that those grown in sand alone (0% Zander) and the leaf number was further significantly increased by incorporation of 30% Zander in the growing medium. At all levels of salinity stress applied, the number of leaves of surviving plants was significantly higher with 10% Zander than with 0% Zander and significantly higher with 30% Zander than with 10% Zander.

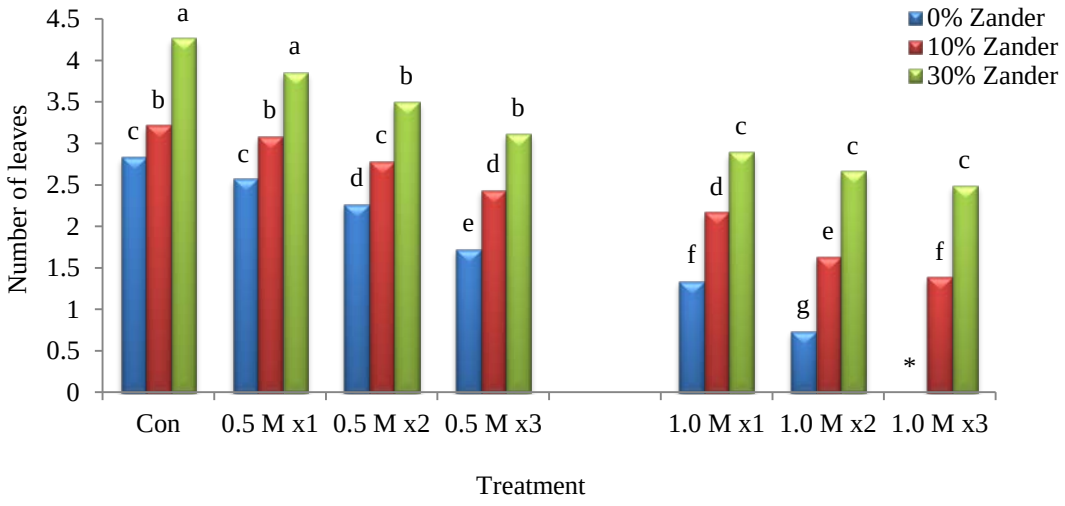


Figure 5.2: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on number of leaves of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

5.3.1.3 Shoot height

Figure 5.3 shows that shoot height of plants grown in sand alone (0% Zander) was not significantly affected by exposure to saline irrigation with 0.5 M NaCl and once with 1.0

M NaCl. Shoot height decreased with increasing concentration of NaCl applied twice and with frequency of irrigation with 1.0 M NaCl. With all frequencies and concentrations of NaCl stress applied, shoot height of plants was significantly greater with 10% Zander than with sand alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander. In the absence of NaCl stress plants grown with 10% Zander had significantly greater shoot height than those grown in the sand alone (0% Zander) and the shoot height was further significantly increased by incorporation of 30% Zander in the grown medium.

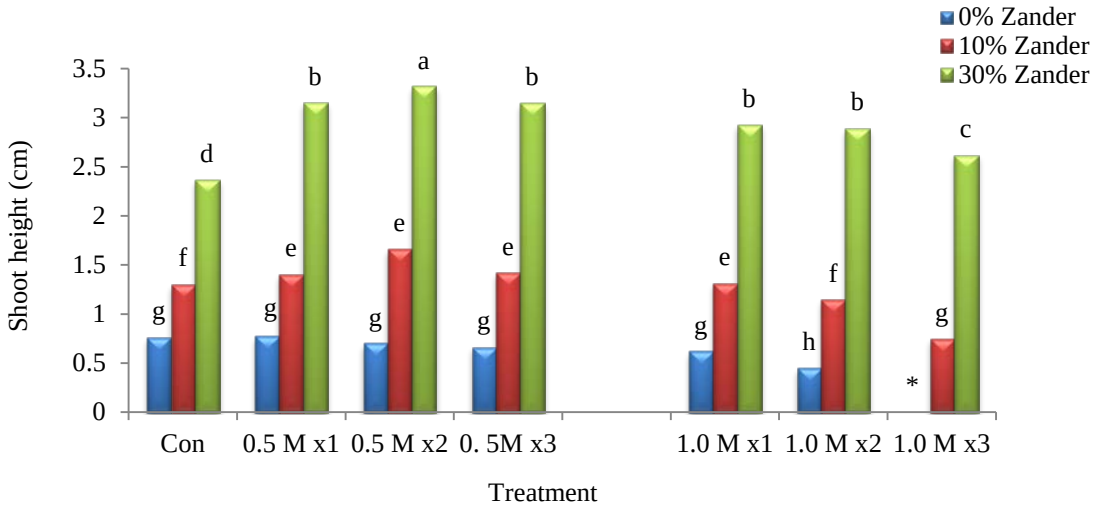


Figure 5.3: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on shoot height of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plant died.

5.3.1.4 Shoot fresh and dry weight and moisture content

Figure 5.4 shows that shoot fresh weight of plants grown in sand alone (0% Zander) was decreased with saline irrigation once or more with 0.5 M NaCl and with 1.0 M NaCl. Fresh weight was lower with increasing concentration of NaCl and with frequency of irrigation with 1.0 M NaCl. Addition of Zander at 10% to the sand significantly increased fresh weight of plants grown without NaCl stress, and this was

further significantly increased with 30% Zander. With all frequencies and concentrations of NaCl stress applied, fresh weight of plants was significantly greater with 10% Zander than with sand alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander.

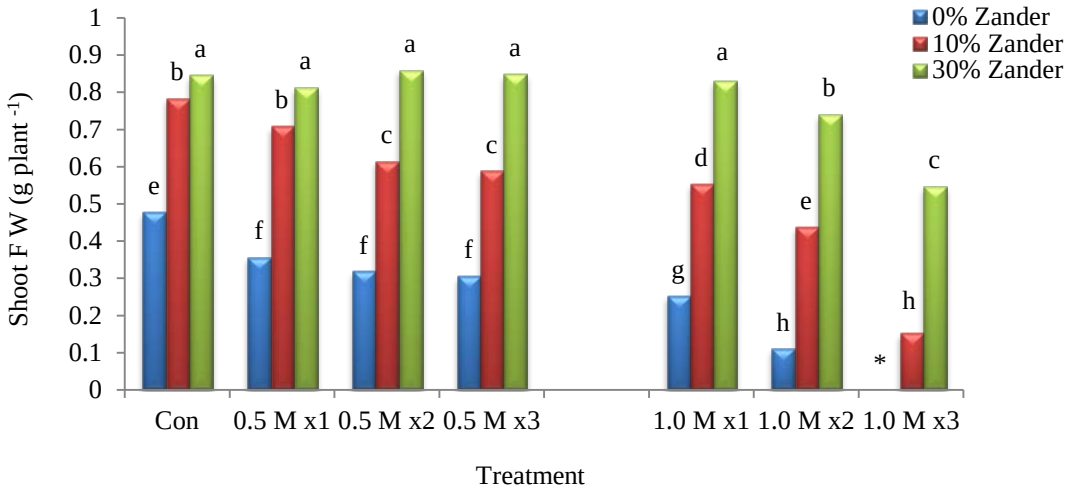


Figure 5.4: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on the shoot fresh weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.*All plants died.

Figure 5.5 shows that in the absence of NaCl stress plants grown with 10% Zander produced significantly more dry weight than those grown in sand alone (0% Zander) and the dry weight was further significantly increased by incorporation of 30% Zander in the growing medium. The dry matter of shoots decreased with increase in salt concentration. When plants were grown in 10% Zander dry weight when irrigated with 0.5 M NaCl once or twice was greater than with unstressed plants in 0% Zander, and this was the case with 30% Zander when irrigation with 0.5 M NaCl and with 1.0 M NaCl, indicating the beneficial effect of Zander on plant dry weight under saline stress. In the absence of NaCl stress plants grown with 10% Zander increased significantly shoot dry weight than those grown in the sand alone (0% Zander) and the shoot dry weight was further significantly increased by incorporation of 30% Zander in the grown medium.

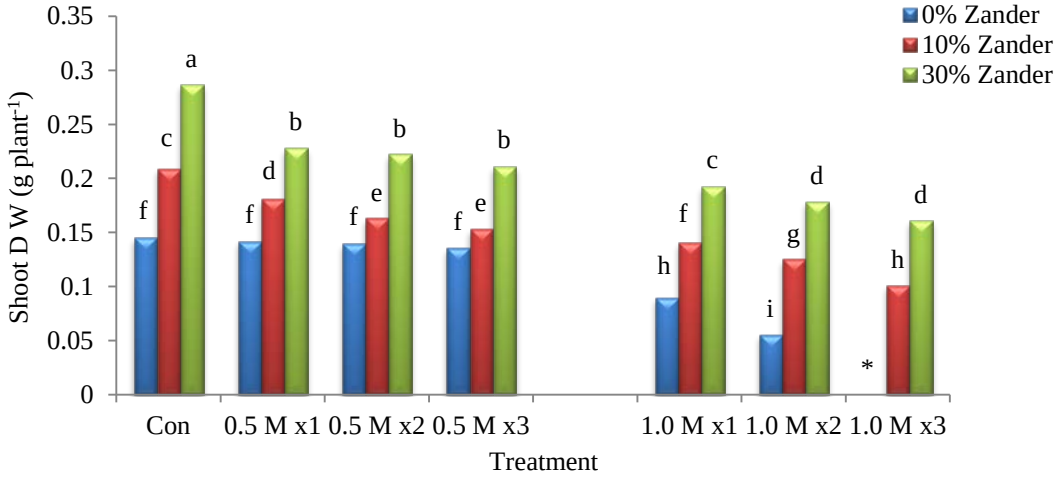


Figure 5.5: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml 0.5 or 1.0 M NaCl) on the shoot dry weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.*All plants died.

Shoot moisture content of plants grown in sand alone (0% Zander) decreased with exposure to saline irrigation two or three times with 0.5 M NaCl and twice or more with 1.0 M NaCl (Figure 5.6). Shoot moisture was lower with increasing concentration when NaCl was applied twice or more and with frequency of irrigation. Moisture content of plants grown with Zander 30% was unaffected by 0.5 or 1.0 M NaCl while that of plants grown with 10% Zander with 1.0 M NaCl applied was significantly reduced.

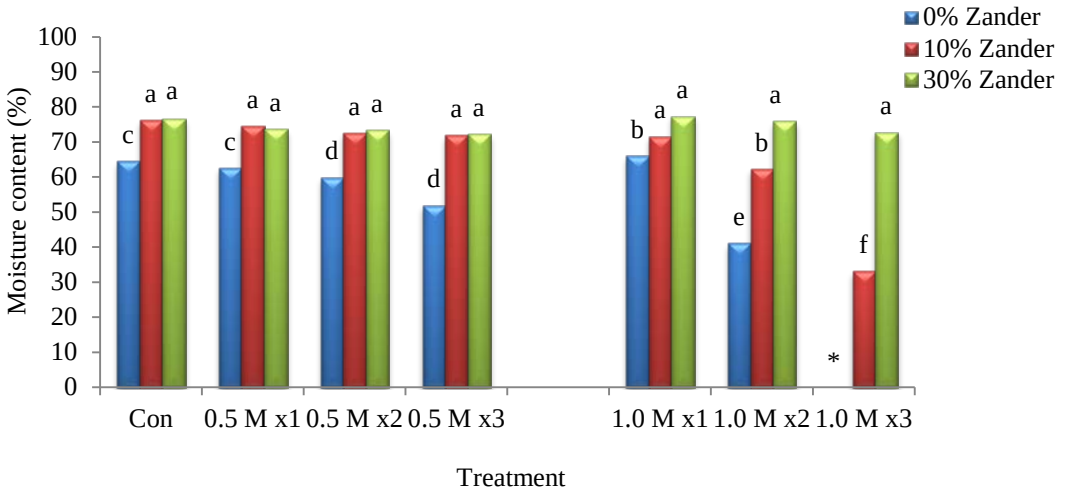


Figure 5.6: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml 0.5 or 1.0 M NaCl) on the shoot moisture content of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

5.3.1.5 Root fresh and dry weight and moisture content

Irrigation with 1.0 M NaCl in sand more then once significantly decreased the root fresh weight (Figures 5.7). Addition of Zander at 10% to the sand significantly increased root fresh weight of plants grown without NaCl stress, and this was further significantly increased with 30% Zander. With NaCl stress applied root fresh weight of plants grown with 10% Zander was significantly greater than with sand alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander.

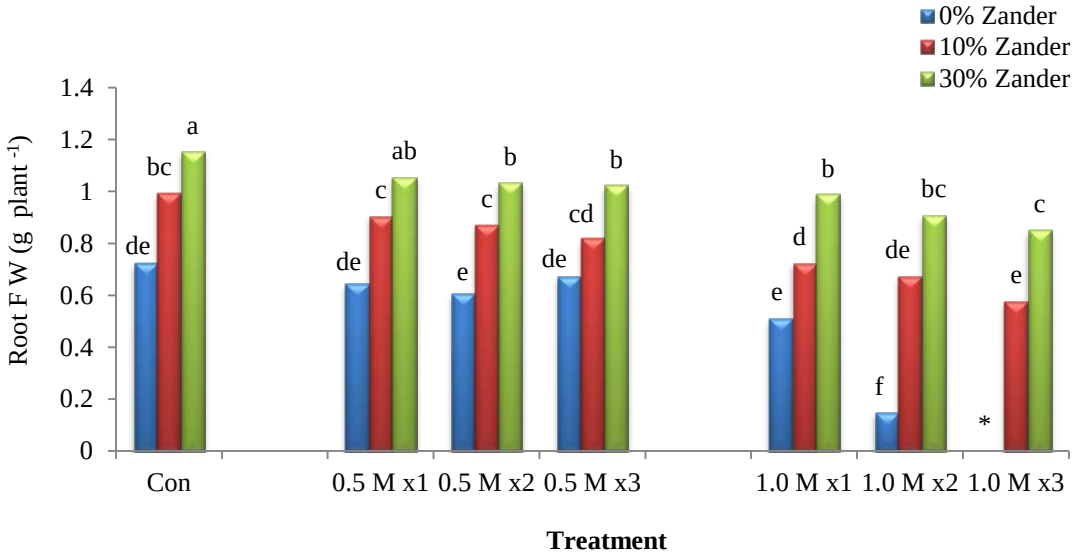


Figure 5.7 Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml 0.5 and 1.0 M NaCl) on the root fresh weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.*All plants died.

Figure 5.8 shows that irrigation with 0.5 M NaCl more than twice or 1.0 M NaCl significantly decreased root dry weight of plants grown in sand alone (0% Zander). Root dry weight was lower with increasing concentration of NaCl and with frequency of irrigation. The highest value of root dry weight was recorded with 30% Zander and lowest with the control, root dry weights achieved for 30% Zander were significant higher than 10% Zander and the lowest weight with 0% Zander.

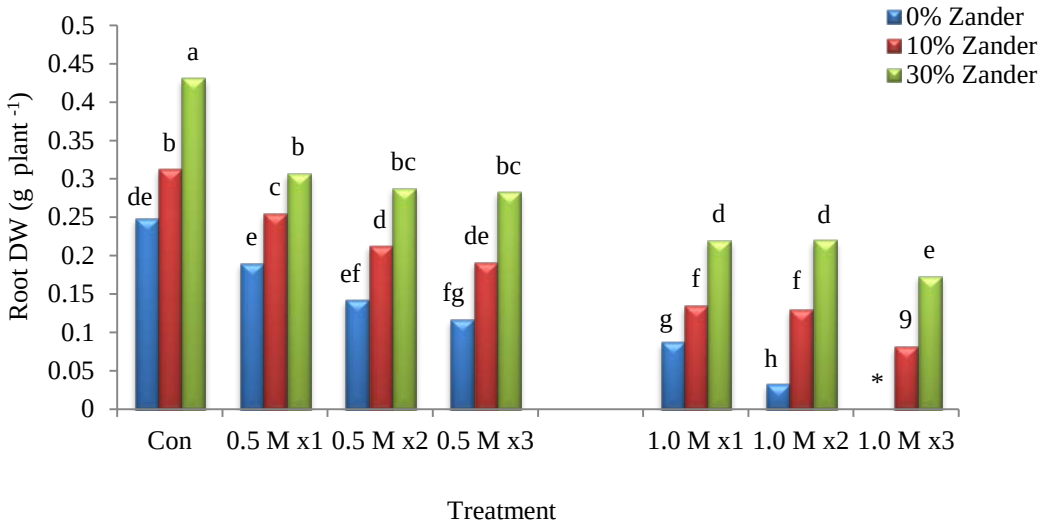


Figure 5.8: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml 0.5 and 1.0 M NaCl) on the root dry weight *Acacia saline* grown in medium with three different concentrations of Zander in the greenhouse *all plants died. Means with the same letter are not at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Figure 5.9 shows moisture content of roots of plants grown in sand alone (0% Zander) increased with exposure to saline irrigation with 0.5 M and 1.0 M NaCl. Moisture content was higher with increasing concentration of NaCl and with frequency of irrigation with 0.5 M NaCl. Addition of Zander at 10% to the sand significantly increased moisture of root of plants grown without NaCl stress and with 1.0 M NaCl.

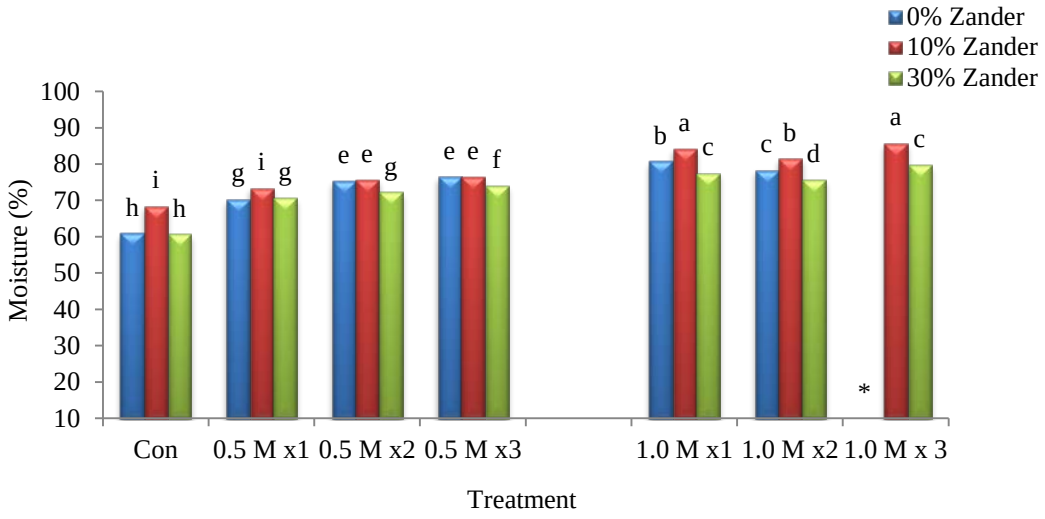


Figure 5.9: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml 0.5 or 1.0 M NaCl) on the root moisture content of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse. Means with the same letter is not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

5.3.2 Mineral content in the greenhouse

5.3.2.1 Mineral content of Zander

The physical and chemical characteristics properties of Zander are shown as the mean of three replicates in Table 5.2

Table 5.2 Mean physical and chemical characteristics of Zander

			Concentration (mg kg^{-1})					
pH	OM%	N%	K	Ca	Na	Mg	Fe	P
6.8-6.9	78-88	1.88	236.5	10144.9	47.3	382.3	4147.6	330.3

The general analysis of Zander shows it has a pH 6.8-6.9 which is favourable to plant growth due to nutrient availability for plants in this range.

Zander is non-saline and has an electrical conductivity of EC 1.04-1.08 dS m^{-1}. Zander contains a high percentage of organic matter, 78- 88%, and also tends to have high cation exchange capacity. Total nitrogen content which was measured by Kjeldahl is 1.88% which is very good for plant growth. The cation group of major nutrients are positively charged ions (Ca^{++}, Mg^{++}, K^{+} and Na^{+}). In Table 5.2 the cation content of Zander is shown to be large. Zander has high moisture content which averaged 35%

5.3.2.2 Mineral content of plants of *Acacia saligna* in the greenhouse

Table 5.3 shows that Na^{+} concentration of plants grown in sand alone increased with increased saline irrigation with 0.5 or 1.0 M NaCl compared with unstressed plants. The Na^{+} concentration of plants increased with increasing concentration and frequency of NaCl irrigation. In the absence of NaCl stress there was no significant difference in the

Na^+ concentration of between plants grown with 0% Zander and those grown with 10% or 30% Zander. When plants were grown in the medium with 30% Zander Na^+ concentration of plants was higher with saline stress. With the highest stress applied 1.0 M NaCl three times, Na^+ concentration was significantly higher with 30% Zander than with 10% Zander and all plants died with 0% Zander (Table 5.3).

Table 5.3: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on Na^+ of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	Concentration of Na^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	133.9 ^{i}	75.8 ^{i}	93.2 ^{i}
0.5 M x 1	2503.1 ^{h}	2081.2 ^{h}	2154.3 ^{h}
0.5 M x 2	4845.9 ^{g}	6350.8 ^{f}	7087.4 ^{e}
0.5 M x 3	7115.8 ^{e}	8057.9 ^{d}	8472.0 ^{cd}
1.0 M x 1	5102.1 ^{g}	5503.2 ^{g}	7157.2 ^{e}
1.0 M x 2	8499.9 ^{cd}	8478.9 ^{cd}	10376.5 ^{b}
1.0 M x 3	*	10051.5 ^{b}	12362.9 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Table 5.4 shows that irrigation with 0.5 M or 1.0 NaCl more than once significantly decreased Ca^{++} concentration of plants compared with unstressed plants. In the absence of NaCl stress Ca^{++} concentration in plants grown with 10% Zander was significantly greater than those grown in sand alone (0% Zander) and Ca^{++} concentration was further significantly increased by incorporation of 30% Zander in the medium. At all level of salinity stress applied Ca^{++} concentration of plants was significantly higher with 10% and 30% Zander than with 0% Zander. However, the difference in Ca^{++} concentration between plants grown in 10% Zander and those grown in 30% Zander was rarely significant. When plants were grown in the medium with 30% Zander, the Ca^{++} concentration of plants was higher with saline stress than Ca^{++} concentration of plants grown in sand alone and without stress.

Table 5.4: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on Ca^{++} of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	Concentration of Ca^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	2771.2 ^{e}	5490.2 ^{b}	6330.4 ^{a}
0.5 M x 1	2534.9 ^{e}	4678.6 ^{bc}	6242.2 ^{ab}
0.5 M x 2	2355.1 ^{f}	4395.6 ^{c}	5031.9 ^{bc}
0.5 M x 3	1364.0 ^{g}	3407.9 ^{d}	4766.4 ^{bc}
1.0 M x 1	2397.4 ^{f}	3725.9 ^{cd}	4295.4 ^{c}
1.0 M x 2	1099.2 $^{f g}$	3629.0 ^{cd}	4237.7 ^{c}
1.0 M x 3	*	3532.50 ^{d}	3958.7 ^{d}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

K^+ concentration of plants irrigated with 1.0 M NaCl more than once was significantly decreased in the plants compared with unstressed plants (Table 5.5). In the absence of NaCl stress K^+ concentration of plants grown with 10% Zander was significantly greater than that of plants grown in sand alone (0% Zander) and K^+ concentration was further significantly increased by incorporation of 30% Zander in the growing medium. With all levels of salinity stress applied, the K^+ concentration of plants was significantly higher with 10% Zander than with 0% Zander and significantly higher with 30% Zander than with 10% Zander. The only exception to this was the lack of significant difference between 10% and 30% Zander treatment with three times irrigation with 1.0 M NaCl. When plants were grown in medium with 30% Zander, the K^+ concentration was significantly higher than the K^+ concentration of plants grown in sand without salinity stress (Table 5.5).

Table 5.5: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on K^+ of *Acacia saline* grown in medium with three different concentrations of Zander in the greenhouse

	Concentration of K^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	3998.2 ^{de}	6936.7 ^{c}	11438.7 ^{a}
0.5 M x 1	3998.2 ^{e}	6767.2 ^{cd}	10009.6 ^{ab}
0.5 M x 2	3371.1 ^{ef}	6254.1 ^{d}	7866.10 ^{b}
0.5 M x 3	2788.2 ^{ef}	5328.7 ^{d}	7148.8 ^{bc}
1.0 M x 1	2713.4 ^{ef}	5374.2 ^{d}	7786.8 ^{bc}
1.0 M x 2	1853.2 ^{f}	4934.6 ^{de}	6267.7 ^{c}
1.0 M x 3	*	4257.9 ^{de}	4995.5 ^{d}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Table 5.6 shows that Mg^{++} concentration of plants grown in sand alone (0% Zander) was decreased with all levels of NaCl irrigation compared with the control. In the absence of NaCl stress Mg^{++} concentration of plants grown with 10% Zander was significantly greater than that of plants grown in sand alone (0% Zander) and Mg^{++} concentration was further significantly increased by incorporation of 30% Zander in the medium. With all frequencies and concentrations of NaCl stress applied Mg^{++} concentration of plants was significantly greater with 10% Zander than with sand alone and significantly greater with 30% Zander than with 10% Zander except with 1.0 M NaCl once. When plants were grown in medium with 30% Zander, the concentration of Mg^{++} was higher at all levels of saline stress than the Mg^{++} concentration in the soil alone and without stress. When plants were grown in 10% Zander Mg^{++} concentration in plants was greater than with unstressed plants in 0% Zander except with 0.5 M three times.

Table 5.6: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on Mg^{++} of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	Concentration of Mg^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	664.9 ^{f}	1416.2 ^{c}	1874.3 ^{a}
0.5 M x 1	493.7 ^{g}	1214.0 ^{cd}	1706.0 ^{b}
0.5 M x 2	399.7 ^{g}	1022.9 ^{d}	1746.9 ^{ab}
0.5 M x 3	281.1 ^{i}	736.7 ^{f}	1383.2 ^{c}
1.0 M x 1	376.6 ^{g}	837.1 ^{d}	1152.1 ^{d}
1.0 M x 2	249.1 ^{i}	798.2 ^{e}	1142.3 ^{cd}
1.0 M x 3	*	701.4 ^{e}	1122.8 ^{d}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Table 5.7 shows that phosphorus concentration of plants grown with 0% Zander decreased with increasing concentration of NaCl but not with number of applications. In the absence of NaCl stress P concentration of plants grown with 10% Zander was significantly greater than in plants grown in sand alone (0% Zander) and P concentration was further significantly increased by incorporation of 30% Zander. When plants were grown in the medium with 10% Zander P concentration was greater than with unstressed plants in 0% Zander for those plants treated with 0.5 M saline solution but not when treated with 1.0 M NaCl.

Table 5.7: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on P of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	Concentration of P (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	328.9 ^{d}	635.1 ^{bc}	821.1 ^{a}
0.5 M x 1	324.6 ^{d}	614.8 ^{bc}	747.1 ^{ab}
0.5 M x 2	310.9 ^{d}	549.1 ^{c}	685.5 ^{b}
0.5 M x 3	266.1 ^{de}	478.1 ^{c}	623.8 ^{bc}
1.0 M x 1	166.7 ^{e}	448.8 ^{cd}	560.6 ^{bc}
1.0 M x 2	123.9 ^{ef}	328.3 ^{d}	431.7 ^{cd}
1.0 M x 3	*	264.6 ^{de}	343.8 ^{d}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Table 5.8 shows that Fe^{++} concentration of plants irrigated with 0.5 M or 1.0 M NaCl significantly increased compared with unstressed plants without Zander in the medium. In the absence of NaCl Fe^{++} concentration of plants grown with 0%, 10% or 30% Zander was not significantly different. At all levels of salinity stress applied, Fe^{++} concentration in plants was significantly higher with 30% than with 0% Zander and significantly higher with 30% than 10% Zander except with 1.0 M NaCl applied more than once.

Table 5.8: Effect of NaCl (0, 1, 2, or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on Fe^{++} of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	Concentration of Fe^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	91.5 ^{i}	87.9 ^{i}	99.8 ^{i}
0.5 M x 1	100.8 ^{f}	112.3 ^{f}	148.1 ^{e}
0.5 M x 2	114.9 ^{f}	152.4 ^{e}	194.3 ^{dc}
0.5 M x 3	116.5 ^{f}	163.4 ^{e}	200.5 ^{c}
1.0 M x 1	123.0 ^{ef}	180.3 ^{d}	217.9 ^{bc}
1.0 M x 2	198.4 ^{cd}	228.8 ^{b}	239.8 ^{ab}
1.0 M x 3	*	252.6 ^{ab}	308.7 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

With all frequencies of NaCl stress applied K^{+}/ Na^{+} ratio of plants was greater with 10% Zander than with the sand alone and greater with 30% than with 10% Zander. Irrigation with 0.5 M NaCl or 1.0 M NaCl drastically decreased K^{+}/ Na^{+} in the plants relative to control (0% Zander). With salinity stress the K^{+}/ Na^{+} ratio was higher with 10% and 30% Zander then with sand alone. Addition of Zander at 10% to the sand increased K^{+}/ Na^{+} of plants grown without NaCl stress, and this was further increased with 30% Zander. At all levels of salinity stress applied, K^{+}/ Na^{+} of plants was higher with 10% than with 0% Zander and higher with 30% than with 10% Zander (Table 5.9).

Table 5.9: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on K^+/ Na^+ ratio of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	K^+ /Na^+		
NaCl	0% Zander	10% Zander	30% Zander
Control	29.85	91.51	122.73
0.5 M x 1	1.60	3.25	4.64
0.5 M x 2	1.44	0.98	1.11
0.5 M x 3	0.39	0.66	0.84
1.0 M x 1	0.53	1.02	1.09
1.0 M x 2	0.22	0.58	0.60
1.0 M x 3	*	0.42	0.48

* All plants died

Irrigation with 0.5 M NaCl or 1.0 M NaCl drastically reduced Ca^{++}/Na^+ ratio compared with that of unstressed plants. Ca^{++}/Na^+ ratio decreased with increasing concentration and frequency of NaCl irrigation. In the absence of NaCl stress Ca^{++}/Na^+ ratio was highest with 10% Zander and lower with 30% and 0% Zander. When plants were grown in medium with 30% Zander the Ca^{++}/Na^+ ratio was greater than in plants grown in 0% or 10% Zander when irrigated with 0.5 M NaCl or 1.0 M once. Plants grown in 10% Zander with salinity stress had greater Ca^{++}/ Na^+ ratio (Table 5.10).

Table 5.10: Effect of NaCl (0, 1, 2, or 3 irrigations of 50 ml with 0.5 M or 1.0 M NaCl) on Ca^{++} /Na^+ ratio of *Acacia saligna* grown in medium with three different concentrations of Zander in the greenhouse

	Ca^{++} /Na^+		
NaCl	0% Zander	10% Zander	30% Zander
Control	20.7	72.4	67.9
0.5 M x 1	1.01	2.24	2.98
0.5 M x 2	0.33	0.69	0.71
0.5 M x 3	0.19	0.42	0.56
1.0 M x 1	0.46	0.68	0.60
1.0 M x 2	0.13	0.43	0.40
1.0 M x 3	*	0.35	0.32

* All plants died

5.3.2.3 Mineral content of the medium in the greenhouse

Table 5.11 shows that Na^+ concentration in the medium at the end of the experiment in sand alone increased significantly with increased saline irrigation with 0.5 or 1.0 M NaCl compared with unstressed medium. Na^+ concentration was higher with increasing concentration of NaCl and with frequency of irrigation. Addition of Zander at 10% or 30% to the sand did not significantly affect of Na^+ concentration in medium without NaCl stress, compared to the control without Zander. With frequency and concentration of NaCl stress applied Na^+ concentration was significantly greater with 10% Zander than with sand alone (0% Zander) except with 0.5M NaCl once, and significantly greater with 30% than with 10% Zander.

Table 5.11: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on Na^+ of the medium with three different concentrations of Zander in the greenhouse

	Concentration of Na^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	11.9 ^{i}	7.7 ^{i}	6.2 ^{i}
0.5 M x 1	157.3 ^{k}	182.3 ^{k}	218.3 ^{h}
0.5 M x 2	338.9 ^{j}	499.5 ^{f}	669.9 ^{e}
0.5 M x 3	452.1 ^{f}	687.6 ^{e}	865.9 ^{d}
1.0 M x 1	639.7 ^{e}	848.4 ^{d}	1001.5 ^{c}
1.0 M x 2	826.1 ^{d}	993.8 ^{c}	1245.1 ^{b}
1.0 M x 3	1087.9 ^{c}	1275.4 ^{b}	1462.9 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.12 shows that Ca^{++} concentration in the medium without Zander at the end of the experiment when irrigated with 0.5 M or with 1.0 M NaCl was significantly higher than the Ca^{++} concentration in the control (no salt stress). In the absence of NaCl stress the medium with 10% Zander contained significantly more Ca^{++} than with sand alone (0% Zander) and the Ca^{++} concentration was also significantly increased by incorporation of

30% Zander. At all levels of salinity stress Ca^{++} concentration in the medium was significantly higher with 10% and 30% than with 0% Zander.

Table 5.12: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on Ca^{++} of the medium with three different concentrations of Zander in the greenhouse

	Concentration of Ca^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	160.3 ^{h}	395.2 ^{f}	454.1 ^{e}
0.5 M x 1	257.8 ^{g}	441.9 ^{e}	737.3 ^{d}
0.5 M x 2	266.7 ^{g}	454.8 ^{e}	931.3 ^{c}
0.5 M x 3	277.6 ^{g}	469.1 ^{e}	993.2 ^{c}
1.0 M x 1	285.7 ^{g}	457.5 ^{e}	1137.1 ^{b}
1.0 M x 2	320.9 ^{f}	567.7 ^{d}	1179.8 ^{b}
1.0 M x 3	337.1 ^{f}	740.6 ^{d}	1268.2 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.13 shows that K^{+} concentration in the medium at the end of the experiment with sand alone was significantly increased with saline irrigation compared with the control in all treatment except 0.5 M NaCl applied once. However, there was a significant difference between saline treatments. In the absence of NaCl stress, K^{+} concentration in the medium with 10% Zander was greater than those grown in soil alone (0% Zander) and this was further significantly increased by incorporation of 30% Zander. At all levels of salinity stress, K^{+} concentrations were higher with 10% and 30% Zander than in sand alone. K^{+} concentration in the medium with irrigation at 1.0 M NaCl was significantly greater with 10% Zander than with sand alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander. K^{+} concentration in the medium with 10% Zander showed no further significant increase fallowing application of 0.5 M NaCl more than twice or 1.0 M NaCl.

Table 5.13: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on K^+ of the medium with three different concentrations of Zander in the greenhouse

	Concentration of K^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	100.7 ^{h}	114.6 ^{g}	129.6 ^{f}
0.5 M x 1	106.3 ^{h}	125.2 ^{f}	194.7 ^{d}
0.5 M x 2	118.1 ^{g}	143.9 ^{e}	227.7 ^{c}
0.5 M x 3	121.8 ^{f}	150.4 ^{e}	240.1 ^{b}
1.0 M x 1	122.9 ^{f}	152.4 ^{e}	252.9 ^{b}
1.0 M x 2	125.2 ^{f}	156.6 ^{e}	268.6 ^{b}
1.0 M x 3	125.8 ^{f}	158.1 ^{e}	303.9 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Mg^{++} concentration in the medium at the end of the experiment was not significantly altered by frequency of application of NaCl at 0.5 M or 1.0 M with eater 0% or 10% Zander. Furthermore, the addition of 10% Zander did not significantly increase the Mg^{++} in the medium compared to treatment without Zander. Addition of 30% Zander to the medium in conjunction with salinity stress significantly elevated Mg^{++} levels compared to 0% and 10% Zander treatments with application of irrigation of 0.5 M and 1.0 M NaCl. In the absence of NaCl stress, Mg^{++} with 10% Zander was not significantly different compared with (0% Zander).

Table 5.14: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on Mg^{++} of the medium with three different concentrations of Zander in the greenhouse

	Concentration of Mg^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	35.9 ^{c}	38.6 ^{c}	46.2 ^{b}
0.5 M x 1	37.6 ^{c}	38.7 ^{c}	56.2 ^{b}
0.5 M x 2	39.1 ^{c}	38.9 ^{c}	57.9 ^{b}
0.5 M x 3	39.6 ^{c}	39.0 ^{c}	62.4 ^{a}
1.0 M x 1	39.6 ^{c}	39.0 ^{c}	67.0 ^{a}
1.0 M x 2	39.9 ^{c}	39.0 ^{c}	67.9 ^{a}
1.0 M x 3	40.0 ^{c}	39.3 ^{c}	70.4 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.15 shows that in the absence of Zander P concentration in the medium at the end of the experiment was significantly increased with more than on application of 0.5 M NaCl and application of 1.0 M NaCl at any level, compared to unstressed control. However, three application of 0.5 M NaCl did not significantly alter P Compared to two applications nor did application of 1.0 M NaCl. In the absence of NaCl stress P concentration in the medium with 10% Zander was significantly greater than in sand alone (0% Zander) and the P concentration was further significantly increased by incorporation of 30% Zander. With saline stress P was significantly greater with 10% Zander than with sand alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander across all NaCl treatments

Table 5.15: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on P of the medium with three different concentrations of Zander in the greenhouse

	Concentration of P (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	85.3 ^{g}	125.4 ^{d}	202.1 ^{c}
0.5 M x 1	85.8 ^{g}	125.4 ^{d}	206.4 ^{b}
0.5 M x 2	86.6 ^{e}	125.5^{d}	208.0 ^{b}
0.5 M x 3	87.0^{e}	125.5 ^{d}	209.0 ^{b}
1.0 M x 1	87.2 ^{e}	125.5 ^{d}	210.9 ^{a}
1.0 M x 2	87.3 ^{e}	125.6 ^{d}	212.1 ^{a}
1.0 M x 3	87.3 ^{e}	125.7 ^{d}	213.3 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.16 shows Fe^{++} concentration in the medium at the end of the experiment Fe^{++} concentration generally increased with increasing saline water irrigation although not always significantly. Addition of Zander at 10% to the sand significantly increased Fe^{++} concentration in the medium without NaCl stress, and this was further increased with 30% Zander compared to 0% and 10% Zander. Across all equivalent frequencies and

concentrations of NaCl stress applied, Fe^{++} was significantly greater with both 10% and 30% Zander in the sand than with sand alone (0% Zander). Fe^{++} concentration treatment with 10% and 30% Zander showed significant increased when irrigated more than once with 0.5 M NaCl or 1.0 M NaCl in the sand alone (0% Zander). With 0% Zander it reqwered two application of 0.5 M NaCl to significantly increase Fe^{++}, but then this was not increased further with application of 1.0 M NaCl three times.

Table 5.16: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on Fe^{++} of the medium with three different concentrations of Zander in the greenhouse

	Concentration of Fe^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	127.3 [g]	180.7 [d]	196.1 [b]
0.5 M x 1	127.6 [f]	184.8 [c]	197.6 [a]
0.5 M x 2	129.5 [e]	184.8 [c]	197.7 [a]
0.5 M x 3	129.6 [e]	184.9 [c]	197.8 [a]
1.0 M x 1	129.5 [e]	184.8 [c]	198.2 [a]
1.0 M x 2	129.6 [e]	184.9 [c]	198.2 [a]
1.0 M x 3	130.7 [e]	185.0 [c]	198.4[a]

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.17 shows that the values of K^+/Na^+ ratio in the medium decreased with increased saline irrigation. Addition of Zander at 10% and 30 % increased K^+/ Na^+ ratio in the medium without NaCl stress. The K^+/ Na^+ ratio in the medium was higher in the control and decreased substantially with saline water irrigation. K^+/Na^+ ratio in the medium with 10% Zander was greater than with 10 % Zander and with sand alone (0% Zander) when irrigation mar then once with 0.5 M NaCl.

Table 5.17: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on K^+ /Na^+ ratio of the medium with three different concentrations of Zander in the greenhouse

	K^+ /Na^+		
NaCl	0% Zander	10% Zander	30% Zander
Control	16.24	14.88	20.90
0.5 M x 1	0.49	0.69	0.98
0.5 M x 2	0.18	0.29	0.34
0.5 M x 3	0.14	0.22	0.17
1.0 M x 1	0.12	0.18	0.15
1.0 M x 2	0.10	0.16	0.13
1.0 M x 3	0.08	0.12	0.11

Table 5.18 shows that irrigation with 0.5 M NaCl or 1.0 M NaCl reduced Ca^{++}/Na^+ ratio compared with that of unstressed medium. Ca^{++}/Na^+ decreased with increasing concentration and frequency of NaCl irrigation. Addition of Zander at 10% to the sand increased Ca^{++}/Na^+ in the medium without NaCl stress, and this was further increased with 30% Zander. In the absence of NaCl stress Ca^{++}/Na^+ was highest with 30% Zander and lower with 10% and 0% Zander.

Table 5.18: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on Ca^{++} /Na^+ ratio of the medium with three different concentrations of Zander in the greenhouse

	Ca^{++}/Na^+		
NaCl	0% Zander	10% Zander	30% Zander
Control	25.85	51.32	73.24
0.5 M x 1	1.18	2.42	3.37
0.5 M x 2	0.40	0.91	1.39
0.5 M x 3	0.32	0.68	1.15
1.0 M x 1	0.29	0.54	1.14
1.0 M x 2	0.26	0.57	0.94
1.0 M x 3	0.23	0.58	0.87

5.3.3 Experiment in the field in Libya

5.3.3.1 Seedling survival

Figure 5.10 shows that survival of plants grown in soil alone (0% Zander) decreased with exposure to saline irrigation once or more with 0.5 M NaCl or 1.0 M NaCl. Survival was lower with increasing concentration of NaCl and with frequency of irrigation. Addition of Zander at 10% to the soil significantly increased survival of plants grown without NaCl treatment, and this was further significantly increased with 30% Zander. With all frequencies and concentrations of NaCl stress applied, survival of plants was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander. Survival of plants grown with Zander at 30% was unaffected by 0.5 M NaCl applied up to three times compared with unstressed plants in the same medium. With the highest stress applied, 1.0 M NaCl three times at 30% Zander survival was approximately 35% whereas no plants survived in soil alone (0% Zander) and 10% Zander.

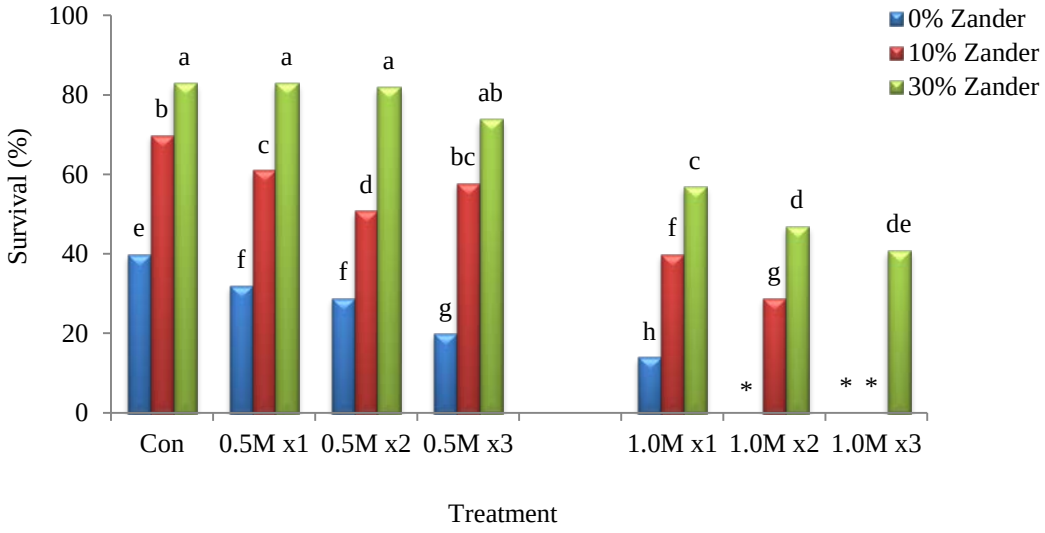


Figure 5.10: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on the survival of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

5.3.3.2 Number of leaves and shoot height

Figure 5.11 shows that irrigation with 0.5 M NaCl more than once or 1.0 M NaCl significantly decreased the number of leaves on surviving plants compared with unstressed ones. Number of leaves decreased with increasing concentration and frequency of NaCl irrigation. Addition of Zander at 10% to the sand significantly increased leaf number of plants grown in the absence NaCl stress, and this was further significantly increased with 30% Zander. At all levels of salinity stress, number of leaves was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with 30% Zander than with 10%.

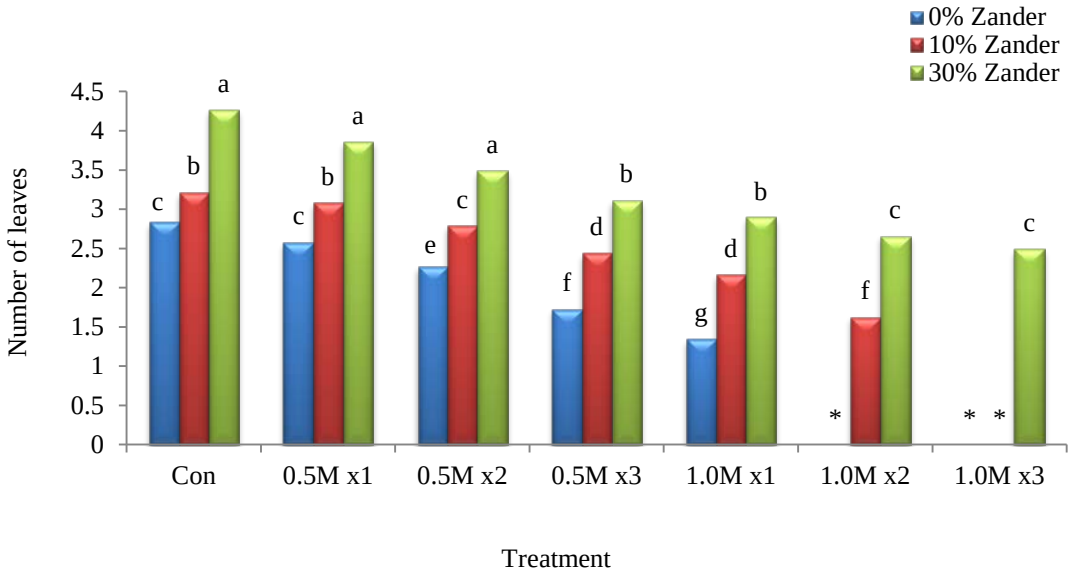


Figure 5.11: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on number of leaves of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Figure 5.12 shows that shoot height was decreased with application of 1.0 M NaCl irrigation more than once. Shoot height was lower with increasing concentration of NaCl and with frequency of 1.0 M irrigation. In the absence of NaCl stress, plants grown with 10% Zander increased shoot height more than those grown in sand alone (0% Zander)

and the shoot height was further significantly increased by incorporation of 30% Zander in grown medium. At all levels of salinity stress applied, the shoot height was significantly higher with 10% Zander than with 0% Zander and significantly higher with 30% than with 10% Zander.

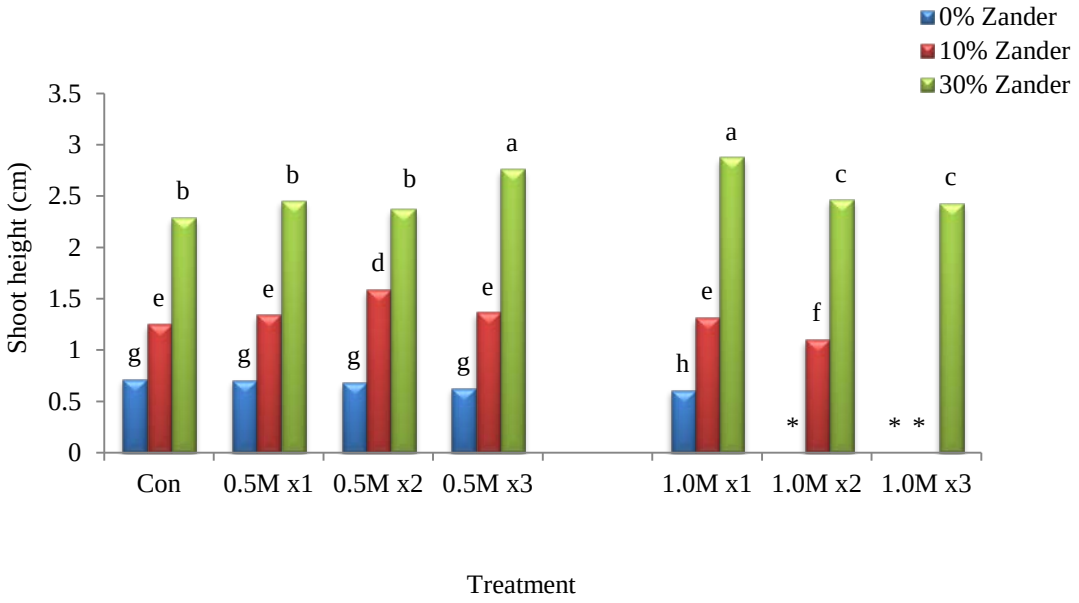
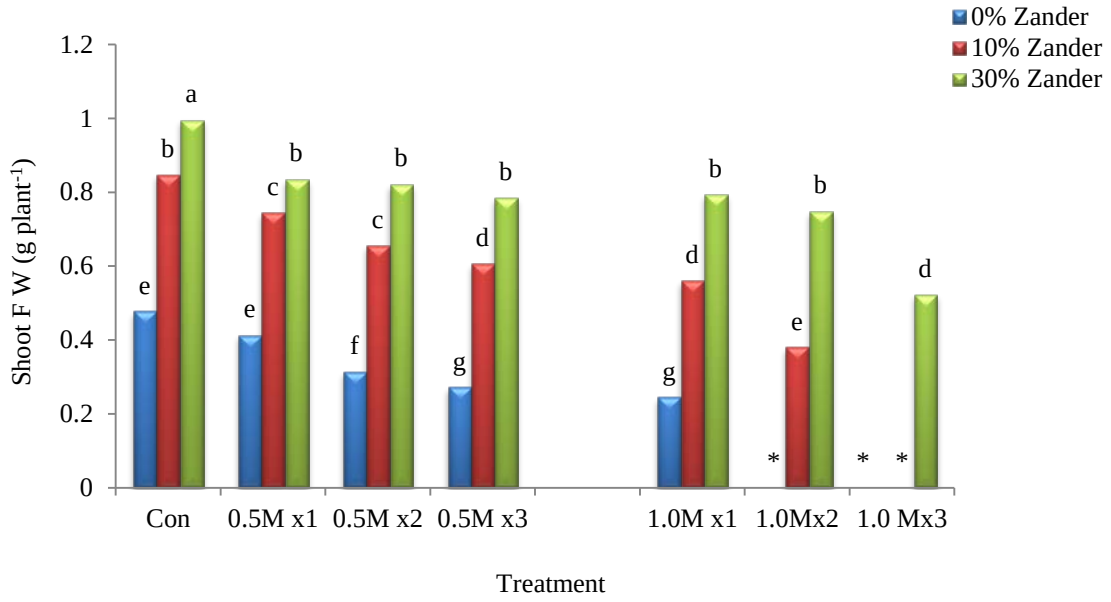


Figure 5.12: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on shoot height of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

5.3.3.3 Shoot fresh weight, dry weight and moisture content

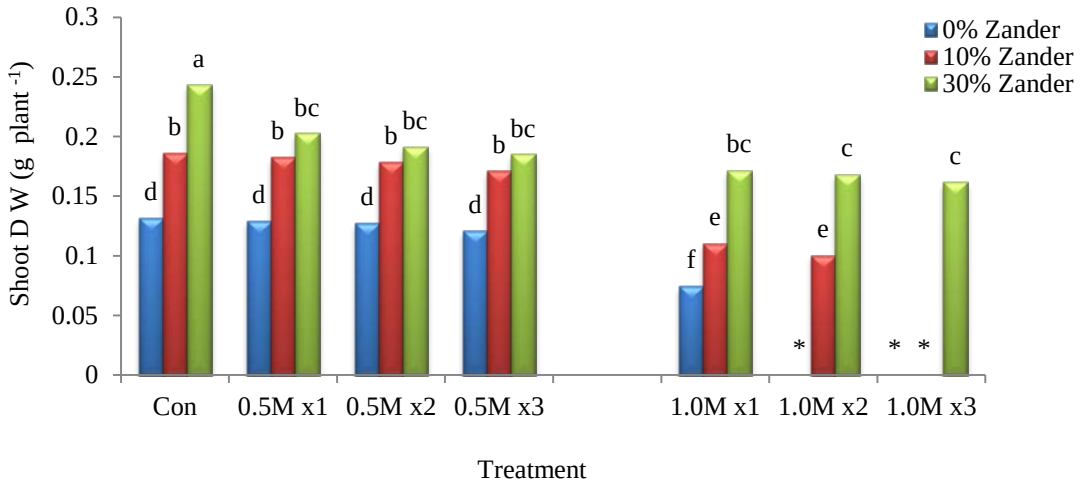
Figure 5.13 shows that shoot fresh weight of plants grown in soil alone (0% Zander) decreased with exposure to saline irrigation once or more using 0.5 M NaCl and once or more with 1.0 M NaCl. Shoot fresh weight was lower with increasing concentration of NaCl and with increasing frequency of irrigation. Addition of Zander at 10% to the soil significantly increased shoot fresh weight of plants grown without NaCl stress, and this was further significantly increased with 30% Zander. With all frequencies and concentrations of NaCl stress applied, shoot fresh weight of plants was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with

30% Zander than with 10% Zander. When plants were grown in 10% Zander shoot fresh weight, when irrigated with 0.5 M or 1.0 M NaCl, was greater than with unstressed plants in 0% Zander and this was higher with 30% Zander than with 10% or 0% Zander, indicating the beneficial effect of Zander on plant fresh weight under saline stress.



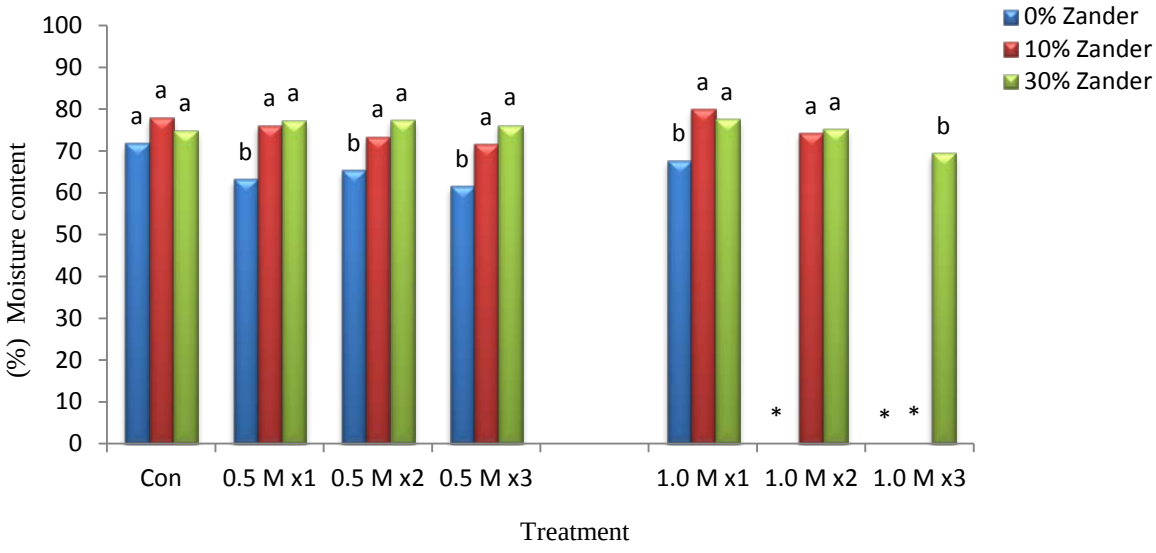
Figurer 5.13: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on shoot fresh weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significant at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Figure 5.14 shows that shoot dry weight of plants grown with soil alone was not affected by 0.5 M NaCl irrigation. Shoot fresh weight of plants grown in soil was decreased with an increase in salt concentration. In the absence of NaCl stress shoot dry weight of plants grown with 10% Zander was greater than those grown in soil alone (0% Zander) and this was further significantly increased by incorporation of 30% Zander in the growing medium. When plants were grown in 10% Zander and irrigated with 0.5 M or 1.0 NaCl of saline solution shoot dry weight was greater than with unstressed plants in 0% Zander. This was also the case with 30% indicating the beneficial effect of Zander on plant dry weight under saline stress.



Figurer 5.14: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on shoot dry weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significant at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

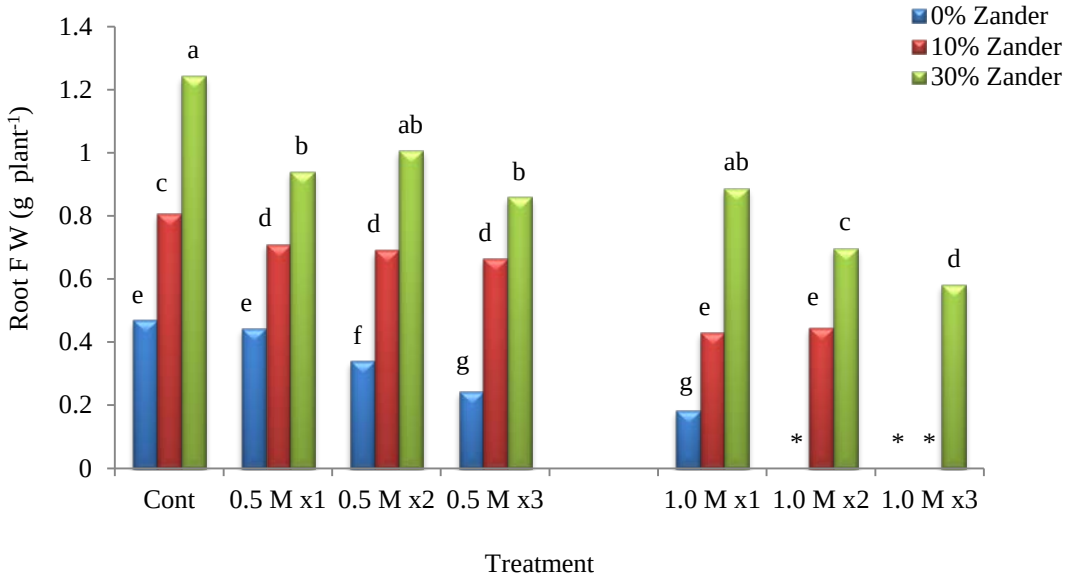
Figure 5.15 shows that the moisture content of shoots grown in the soil alone decreased with saline irrigation. With 10% and 30% Zander, moisture content was not significant affected by irrigation with 0.5 M or with 1.0 M NaCl either once or twice. In the absence of NaCl stress, moisture content was not significantly affected when Zander was added to the soil.



Figurer 5.15: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on shoot moisture content of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significant at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

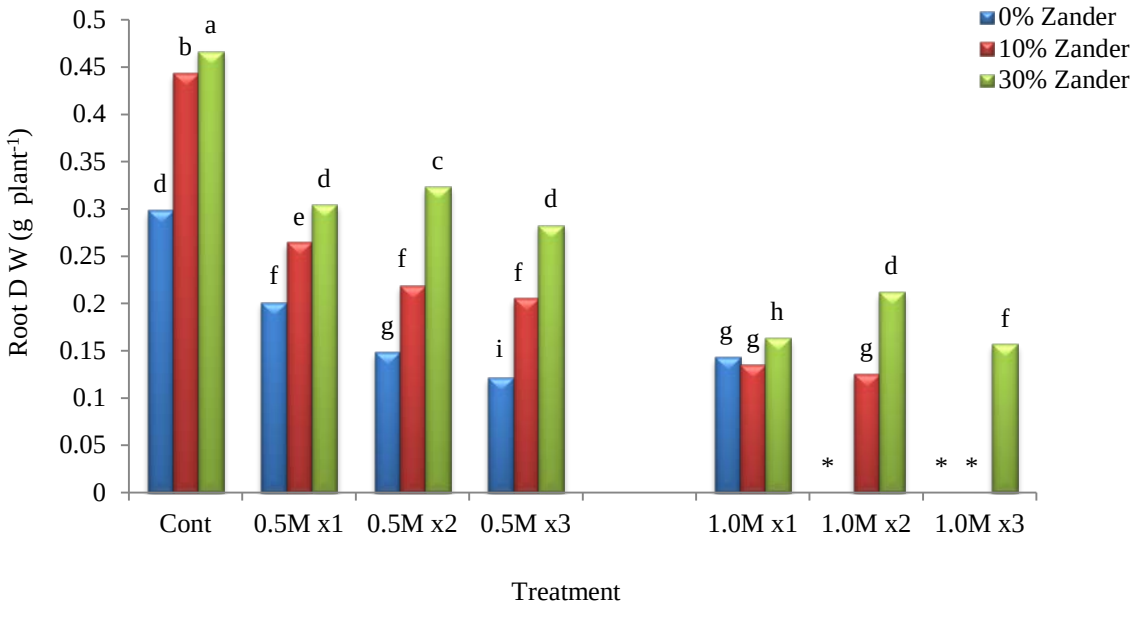
5.3.3.4 Root fresh weight, dry weight and moisture content

Figure 5.16 shows that root fresh weight of plants grown in the soil alone decreased with exposure to saline irrigation two or three times with 0.5 M NaCl and more than once with 1.0 M NaCl. Root fresh weight was lower with increasing concentration and with frequency of irrigation. Addition of Zander at 10% to the soil significantly increased root fresh weight of plants grown without NaCl stress and this was further significantly increased with 30% Zander. With all levels of salinity applied root fresh weight was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander. When plants were grown in 10% Zander, root fresh weight when irrigated with 0.5 M NaCl once or twice was significantly greater than with 0% Zander, and this was the case with 30% Zander. When plants were grown in the medium with 30% Zander, the root fresh weight was higher at all levels of saline stress compared with plants grown in sand alone and without stress.



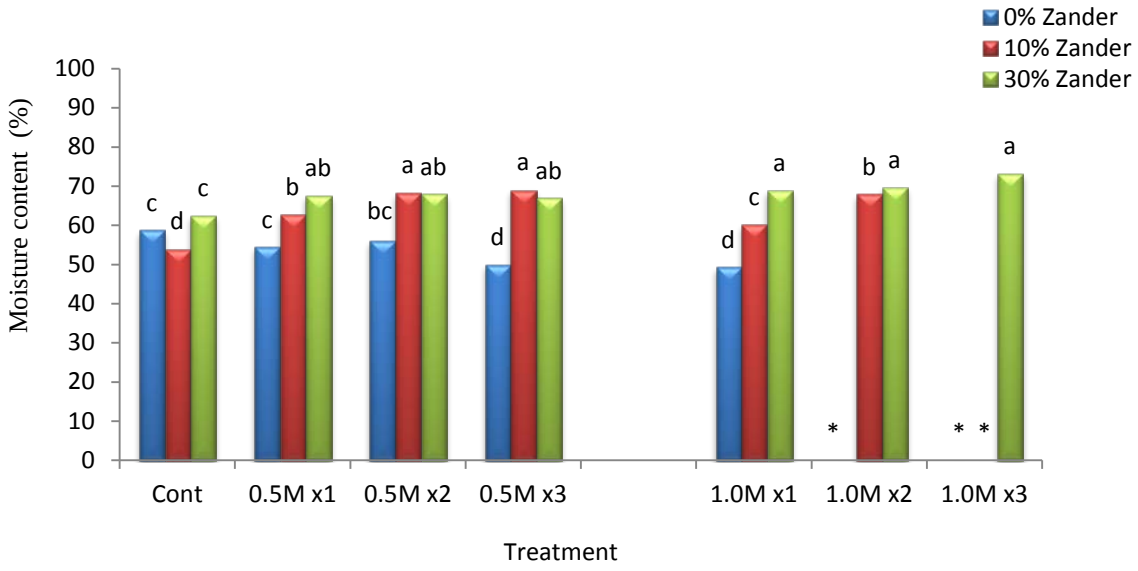
Figurer 5.16: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on root fresh weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significant at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Figure 5.17 shows that root dry weight of plants grown in the soil alone (0% Zander) when irrigated with 0.5 M and 1.0 M NaCl more than once, decreased with increasing concentration and frequency of NaCl irrigation. In the absence of NaCl stress, plants grown in 10% Zander produced more root dry weight than those grown in the soil alone and this was further significantly increased with 30% Zander. With 0.5 M of NaCl stress applied, root dry weight of plants was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with 30% than with 10% Zander. When plants were grown in 10% Zander root dry weight, when irrigated with 0.5 M NaCl once or twice was greater than with 0% Zander, and this was the case with 30% Zander. Root dry weight with 30% Zander when irrigated with 0.5 M NaCl one, two or three times, and with 1.0 M NaCl one was greater than with 10% Zander and with 0% Zander. These indicate the beneficial effect of Zander on root dry weight under saline stress.



Figurer 5.17: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on root dry weight of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significant at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Figure 5.18 shows that moisture content of roots of plants grown in soil alone (0% Zander) decreased with exposure to saline irrigation three times with 0.5 M NaCl and that with two or more irrigations with 1.0 M NaCl all plants died. Moisture content was higher with increasing concentration of NaCl. Addition of Zander at 10% to the soil significantly decreased moisture content of roots of plants grown without NaCl stress, but this was significantly higher with 30% Zander than with 10% Zander and was unchanged compared with the soil alone (0% Zander). At all levels of salinity stress applied, the moisture content of roots of plants was significantly higher with 10% Zander than with 0% Zander. However, with 0.5 M Knack saline irrigation there was no significantly increase in moisture content of roots with 30% compared with10% Zander. When plants were grown in 10% Zander, root moisture content when irrigated with 0.5 NaCl once or twice and three times, was greater than with unstressed plants in 0% Zander and this was also the case for with 30% Zander compared with the control. When plants were irrigation once or more with 1.0 M NaCl moisture content of roots was increased with 30% and 10% Zander while all plants died in the control.



Figurer 5.18: Effect of NaCl (0, 1, 2 or 3 irrigations of 50 ml with 0.5 or 1.0 M NaCl) on root moisture content of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya. Means with the same letter are not significant at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

5.3.4 Mineral content in the field in Libya

5.3.4.1 Mineral content of plants of *Acacia saligna* in the field in Libya

Table 5.19 shows that irrigation with 0.5 M NaCl once more or 1.0 M NaCl, significantly increased Na^+ concentration of plants compared with unstressed plants. Na^+ concentration of plants increased progressively with increased frequency and concentration of NaCl irrigation. Addition of Zander at 10% to the soil did not significantly increase Na^+ of plants grown without NaCl stress, but this was significantly increased with 30% Zander. Across all frequencies and concentrations of NaCl stress applied, Na^+ concentration of plants was significantly greater with 10% Zander than with 0% Zander except with 0.5 M NaCl once, and significantly greater with 30% Zander than with 10% Zander.

Table 5.19: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Na^+ of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Concentration of Na^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	204.7 ^{h}	283.4 ^{h}	396.8 ^{i}
0.5 M x 1	5563.1^{g}	5586.8 ^{g}	6066.7 ^{f}
0.5 M x 2	7363.7 ^{f}	8448.1^{e}	9504.9 ^{d}
0.5 M x 3	9617.3 ^{d}	11066.5 ^{c}	13261.1 ^{b}
1.0 M x 1	8984.8 ^{e}	9291.7 ^{d}	11335.9 ^{c}
1.0 M x 2	*	11804.5 ^{c}	14658.4 ^{b}
1.0 M x 3	*	*	16889.1 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variasnce and using Tukey's test. * All plants died.

Table 5.20 shows that Ca^{++} concentration of plants grown in the soil alone (0% Zander) decreased with increased saline irrigation with 0.5 M NaCl and with 1.0 M NaCl. Ca^{++} was lower with increased concentration of NaCl and with frequency of irrigation. In the

absence of NaCl stress plants grown with 10% Zander had significantly higher Ca^{++} concentration than those grown in soil alone (0% Zander) and the Ca^{++} concentration was further significantly increased by incorporation of 30% Zander in the growing medium. Across all frequencies and concentrations of NaCl stress applied, Ca^{++} was significantly greater with 10% Zander than with 0% Zander, When plants were grown in medium with 30% Zander the Ca^{++} concentration in plants was higher at all levels of saline stress than in plants in grown in soil alone and without stress except 1.0 M NaCl twice or three times with the unstressed control. Ca^{++} concentration was significantly greater with 30% Zander than with 10% Zander, at all saline concentration and at all frequencies of irrigation.

Table 5.20: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Ca^{++} of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Concentration of Ca^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	14049.1 [d]	16913.3 [b]	19567.7 [a]
0.5 M x 1	11975.3 [e]	15200.9 [c]	19641.1 [a]
0.5 M x 2	9526.1 [f]	15158.7 [c]	17143.4 [ab]
0.5 M x 3	8155.9 [g]	10890.5 [e]	15567.7 [c]
1.0 M x 1	9309.4 [f]	13936.2 [d]	16109.4 [ab]
1.0 M x 2	*	9327.5 [f]	14962.7 [d]
1.0 M x 3	*	*	12871.2 [e]

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.* All plants died.

Table 5.21 shows that concentration of K^+ in plants grown in the soil alone (0% Zander) decreased with exposure to all saline irrigation treatments with 0.5 M and once with 1.0 M NaCl. K^+ concentration was lower with increasing concentration of NaCl and with frequency of irrigation. Addition of Zander at 10% to the soil significantly increased concentration of K^+ in plants grown without NaCl stress, and this was further

significantly increased with 30% Zander. Across all frequency and concentrations of NaCl stress applied, K^+ concentration in plants was significantly greater with 10% Zander than with soil alone (0% Zander) with no significant affect with 30% Zander compared with 10% Zander of plants grown in the same medium. K^+ concentration of plants grown with 10% Zander was unaffected by 0.5 M NaCl applied once or twice and with 30% Zander more the once. When plants were grown in 10% Zander K^+ concentration when irrigated with 0.5 M NaCl once or twice was greater than with unstressed plants in 0% Zander, and this was the case with 30% Zander and irrigation with 0.5 M NaCl one, two or three times.

Table 5.21: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on K^+ of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Concentration of K^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	10116 .2 [d]	13973.5 [b]	15765.7 [a]
0.5 M x 1	9660.9 [e]	12721.9 [b]	13197.4 [b]
0.5 M x 2	723.1.6 [f]	11190.3 [c]	11561.5 [c]
0.5 M x 3	5385.7 [h]	10655.7 [d]	11500.2 [c]
1.0 M x 1	6928.3 [g]	9067.7 [e]	10979.6 [d]
1.0 M x 2	*	8080.2 [f]	9116.2 [e]
1.0 M x 3	*	*	8152.2 [f]

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Mg^{++} concentration of plants grown in soil alone (0% Zander) decreased with more the one exposure to saline irrigation with 0.5 M NaCl, or once with 1.0 M NaCl. Mg^{++} concentration was lower with increased concentration of NaCl irrigation. In the absence of NaCl stress, plants grown with 10% Zander had a significantly higher Mg^{++} concentration than those grown in the soil alone (0% Zander) and the Mg^{++} concentration of plants was further significantly increased by incorporation of 30% Zander in the growing medium. When plants were growing in 10% Zander Mg^{++}

concentration when irrigated with 0.5 M NaCl once was greater than Mg^{++} concentration with unstressed plants in 0% Zander, and this was also the case with 30% Zander (Table 5.22).

Table 5.22: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Mg^{++} of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Concentration of Mg^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	2792.9 [c]	3193.9 [b]	3968.1 [a]
0.5 M x 1	2632.1 [c]	3003.4 [b]	3657.3 [a]
0.5 M x 2	2515.7 [d]	2884.8 [c]	3558.8 [b]
0.5 M x 3	2260.4 [d]	2678.7 [c]	2803.6 [c]
1.0 M x 1	1934.3 [e]	2329.8 [d]	3191.7 [b]
1.0 M x 2	*	2199.9 [d]	2752.5 [c]
1.0 M x 3	*	*	2172.8 [d]

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Table 5.23 shows that P concentration of plants grown in the soil alone (0% Zander) was not significantly affected by 0.5 M NaCl but was decreased by 1.0 M NaCl. In the absence of NaCl stress, plants grown with 10% Zander contained significantly more P than those grown in soil alone and the P concentration in plants was further significantly increased by incorporation of 30% in the growth medium. P concentration of plants grown with 10% Zander was decreased by 0.5 M NaCl compared with unstressed plants in the same medium and this case with 10% and 30% Zander. Phosphorus in the plants grown with Zander at 30% and 10% was higher than 0% Zander. For three times treatment with 1.0 M NaCl only plants grown in 30% Zander medium were able to grow. Plants in 0% and 10% Zander all died with 1.0 M NaCl applied three times.

Table 5.23: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on P of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Concentration of P (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	1070.7 ^{cd}	1700.2 ^{b}	2396.1 ^{a}
0.5 M x 1	905.2 ^{cd}	1375.3 ^{bc}	2011.3 ^{ab}
0.5 M x 2	873.5 ^{cd}	1216.1 ^{cd}	1503.6 ^{bc}
0.5 M x 3	1007.1 ^{cd}	936.4 ^{cd}	1279.9 ^{c}
1.0 M x 1	408.1 ^{e}	815.4 ^{d}	1585.8 ^{bc}
1.0 M x 2	*	576.2 ^{d}	1158.1 ^{cd}
1.0 M x 3	*	*	919.1 ^{cd}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. * All plants died.

Table 5.24 shows that Fe^{++} concentration of plants grown in the soil alone (0% Zander) was significantly decreased by 0.5 M NaCl and 1.0 M NaCl. Irrigation with 0.5 M NaCl more than once, or 1.0 M NaCl significantly increased the Fe^{++} in the plants compared with unstressed plants. Fe^{++} increased with increased concentration and frequency of NaCl irrigation. In the absence of NaCl stress plants grown with 10% or 30% Zander contained significantly more Fe^{++} than those grown in the soil alone (0% Zander).

Table 5.24: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Fe^{++} of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Concentration of Fe^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	189.8 ^{f}	219.7 ^{e}	225.6 ^{e}
0.5 M x 1	208.1 ^{e}	243.2 ^{d}	265.1 ^{c}
0.5 M x 2	238.8 ^{cd}	244.8 ^{d}	340.6 ^{b}
0.5 M x 3	247.7 ^{d}	245.4 ^{d}	406.7 ^{ab}
1.0 M x 1	240.9 ^{d}	283.1 ^{bc}	358.1 ^{b}
1.0 M x 2	*	296.5 ^{b}	453.7 ^{a}
1.0 M x 3	*	*	539.8 ^{a}

Means without the same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test. *All plants died.

Tables 5.25 shows that addition of Zander at 10% to the soil decreased the Ca^{++}/Na^{+} ratio of plants grown without NaCl stress, and this was further decreased with 30% Zander. With all frequencies and concentration of NaCl stress applied Ca^{++}/Na^{+} and Ca^{++}/Na^{+} ratio was greater with 10% Zander than with soil alone (0% Zander) and less with 30% Zander. Tables 6.26 shows that addition of Zander at 10% to the soil decreased the K^{+}/Na^{+} ratio of plants grown without NaCl stress, and this was further decreased with 30% Zander. With all levels of Zander K^{+}/Na^{+} ratio was decreased with increased frequency and concentration of irrigation.

Table 5.25: effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Ca^{++}/Na^{+} ratio of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	Ca^{++}/Na^{+}		
NaCl	0% Zander	10% Zander	30% Zander
Control	68.63	59.68	49.31
0.5 M x 1	2.12	2.14	3.23
0.5 M x 2	1.29	1.79	1.80
0.5 M x 3	0.85	0.97	1.17
1.0 M x 1	1.04	1.50	1.42
1.0 M x 2	*	0.79	1.02
1.0 M x 3	*	*	0.76

*All plants died

Table 5.26: effect of NaCl (0, 1, 2or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on K^{+}/Na^{+} ratio of *Acacia saligna* grown in medium with three different concentrations of Zander in the field in Libya

	K^{+}/Na^{+}		
NaCl	0% Zander	10% Zander	30% Zander
Control	49.42	49.30	39.731
0.5 M x 1	1.73	2.27	2.17
0.5 M x 2	0.98	1.32	1.22
0.5 M x 3	0.56	0.96	0.87
1.0 M x 1	0.77	0.97	0.98
1.0 M x 2	*	0.68	0.62
1.0 M x 3	*	*	0.48

*All plants died

5.3.4.2 Mineral content of medium in the field in Libya

Table 5.27 shows that Na^+ concentration in the medium with soil alone (0% Zander) increased significantly with exposure to the saline irrigation. Na^+ concentration in the medium was higher with increasing concentration of NaCl and with frequency of irrigation. Addition of Zander at 10% and 30% to the soil significantly decreased the Na^+ concentration in the medium without NaCl stress. With all frequencies and concentrations of NaCl stress applied, Na^+ concentration in the medium was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander.

Table 5.27: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Na^+ in the medium with three different concentrations of Zander in the field in Libya

	Concentration of Na^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	216.3 ^{h}	164.5 ^{k}	205.4 ^{i}
0.5 M x 1	854.3 ^{g}	1030.6 ^{f}	1360.1 ^{e}
0.5 M x 2	1089.4 ^{f}	1261.4 ^{e}	1840.4 ^{d}
0.5 M x 3	1244.4 ^{ef}	1856.3 ^{d}	2392.8 ^{c}
1.0 M x 1	1076.9 ^{f}	1353.6 ^{e}	2011.1 ^{c}
1.0 M x 2	1369.1 ^{e}	1875.8 ^{d}	2445.4 ^{c}
1.0 M x 3	1967.4 ^{c}	2547.4 ^{b}	2926.7 ^{a}

Means without a same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.28 shows that irrigation with 0.5 M NaCl or 1.0 M NaCl significantly increased the Ca^{++} concentration in the medium when compared with unstressed medium. The Ca^{++} concentration in the medium increased with increasing concentration of NaCl irrigation. The Ca^{++} concentration following application of 1.0 M NaCl were all significantly higher with 0.5 M NaCl, regardless of number of application In the absence of NaCl stress, Ca^{++} concentration in the medium with 10% Zander was significantly

higher than in the soil alone (0% Zander) and the Ca^{++} was further significantly increased by incorporation of 30% Zander in the growing medium. In the medium with 30% Zander the Ca^{++} concentration was high or higher at all levels of saline stress than in the medium of soil alone and without stress.

Table 5.28: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Ca^{++} in the medium with three different concentrations of Zander in the field in Libya

	Concentration of Ca^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	1026.1 ^{k}	1796.1 ^{h}	2930.5 ^{e}
0.5 M x 1	1059.9 ^{j}	1875.6 ^{g}	3036.9 ^{d}
0.5 M x 2	1088.2 ^{j}	1881.0 ^{g}	3055.0 ^{c}
0.5 M x 3	1096.5 ^{j}	1914.0 ^{g}	3105.0 ^{b}
1.0 M x 1	1099.2 ^{i}	1947.7 ^{f}	3178.2 ^{a}
1.0 M x 2	1110.5 ^{i}	1959.8 ^{f}	3183.3 ^{a}
1.0 M x 3	1115.5 ^{i}	1979.5 ^{f}	3212.6 ^{a}

Means without a same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.29 shows that with 0% Zander irrigation with 0.5 M or 1.0 M NaCl significantly increased K^{+} in the medium compared with unstressed medium. K^{+} concentration increased significantly with concentration of NaCl irrigation, but not with just one application of 0.5 M NaCl. In the medium with 30% Zander, K^{+} concentration was as high or higher at all levels of saline stress as in the medium with soil alone and without stress. Addition of Zander at 10% to the soil significantly increased K^{+} concentration in the medium without NaCl stress. When soil was mixed with 10% Zander K^{+} concentration when irrigated with 0.5 M NaCl once or more was greater than with unstressed medium in 0% Zander, and this also was the case with 30% Zander (Table 5.30).

Table 5.29: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on K^+ in the medium with three different concentrations of Zander in the field in Libya

	Concentration of K^+ (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	129.4 ^{j}	185.5 ^{i}	212.9 ^{e}
0.5 M x 1	149.4 ^{j}	257.0 ^{g}	326.3 ^{d}
0.5 M x 2	173.3 ^{i}	267.8 ^{f}	337.9 ^{c}
0.5 M x 3	180.9 ^{i}	269.7 ^{f}	363.2 ^{b}
1.0 M x 1	180.8 ^{i}	316.3 ^{ef}	420.6 ^{a}
1.0 M x 2	190.1 ^{h}	319.9 ^{d}	425.8 ^{a}
1.0 M x 3	194.1 ^{h}	337.0 ^{c}	432.7 ^{a}

Means without a same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.30 shows that irrigation with 0.5 M NaCl or 1.0 M NaCl significantly increased the Mg^{++} in the medium compared with unstressed medium. Mg^{++} in the medium increased with increasing concentration of NaCl irrigation. In the absence of NaCl stress, medium with 10% Zander produced significantly more Mg^{++} than with soil alone (0% Zander) and the Mg^{++} with 30% Zander was greater than with 10% Zander**.** In the medium with 30% Zander the Mg^{++} concentration was high or higher at all levels of saline stress than in the medium of soil alone and without stress.

Table 5.30: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Mg++ in the medium with three different concentrations of Zander in the field in Libya

	Concentration of Mg^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	199.4 ^{i}	355.8 ^{g}	456.7 ^{d}
0.5 M x 1	205.1 ^{i}	369.9 ^{f}	481.7 ^{c}
0.5 M x 2	210.3 ^{h}	371.7 ^{f}	481.0 ^{c}
0.5 M x 3	212.3 ^{h}	374.3 ^{f}	495.3 ^{b}
1.0 M x 1	213.6 ^{h}	385.1 ^{e}	507.2 ^{a}
1.0 M x 2	216.2 ^{h}	385.8 ^{e}	508.3 ^{a}
1.0 M x 3	220.1 ^{h}	390.4 ^{e}	515.0 ^{a}

Means without a same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.31 shows that concentration of P in the medium without Zander (0%) soil alone was significantly increased by 0.5 M and 1.0 M NaCl irrigated. Addition of Zander at 10% to the soil significantly increased phosphorus in medium without NaCl stress and this was further significantly increased with 30% Zander. With all frequencies and concentrations of NaCl stress applied phosphorus was significantly greater with 10% Zander than with soil alone (0% Zander) and significantly greater with 30% Zander than with 10% Zander. With the highest stress applied with 1.0 M NaCl irrigation phosphorus was significantly greater with 30% Zander than 0% and 10% Zander and without stress.

Table 5.31: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on P in the medium with three different concentrations of Zander in the field in Libya

	Concentration of P (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	8.6 ^{h}	11.4 ^{g}	21.1 ^{f}
0.5 M x 1	11.2 ^{g}	20.5 ^{f}	38.3 ^{c}
0.5 M x 2	12.9 ^{g}	21.9 ^{f}	42.9 ^{b}
0.5 M x 3	13.3 ^{g}	24.2 ^{e}	48.1 ^{b}
1.0 M x 1	14.5 ^{f}	27.9 ^{d}	53.0 ^{a}
1.0 M x 2	15.0 ^{f}	28.6 ^{d}	54.4 ^{a}
1.0 M x 3	17.0 ^{f}	29.8 ^{d}	57.2 ^{a}

Means without a same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.32 shows that Fe^{++} in the soil alone (0% Zander) unaffected with saline water irrigation with 0.5 M NaCl or with 1.0 M NaCl. Addition of Zander to the soil significantly increased of Fe^{++} in the medium in the absence of saline stress. With all frequencies and concentrations of NaCl stress applied, Fe^{++} was significantly greater with 10% Zander than with soil alone, Fe^{++} was significantly greater with 30% Zander than with 10% Zander.

Table 5.32: Effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on Fe^{++} in the medium with three different concentrations of Zander in the field in Libya

	Concentration of Fe^{++} (mg kg^{-1})		
NaCl	0% Zander	10% Zander	30% Zander
Control	499.8 [e]	584.4 [c]	996.2 [b]
0.5 M x 1	500.1 [e]	586.1 [c]	998.2 [b]
0.5 M x 2	501.2 [e]	586.1 [c]	998.4 [b]
0.5 M x 3	501.4 [e]	585.7 [c]	1000.5 [a]
1.0 M x 1	501.8 [e]	589.0 [c]	1006.9 [a]
1.0 M x 2	502.8 [e]	588.4 [c]	1007.1 [a]
1.0 M x 3	503.8 [e]	590.7 [c]	1008.5 [a]

Means without a same letter in common differ significantly at $p \leq 0.05$ based on two-way analysis of variance and using Tukey's test.

Table 5.33 shows that addition of Zander at 10% to the soil increased Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio of the medium without NaCl stress, and this was also increased with 30% Zander. Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio in the soil alone (0% Zander) was low and increased substantially in a media with 10% and 30% Zander. Ca^{++}/Na^{+} ratio in the medium with 30% Zander was greater than with 0% Zander and with 10% Zander for all frequencies and concentrations of NaCl.

Table 5.33: effect of NaCl (0, 1, 2 or 3 irrigation of 50 ml with 0.5 M or 1.0 M NaCl) on K^{+}/Na^{+} and Ca^{++}/Na^{+} ratios in the medium with three different concentrations of Zander in the field in Libya

	K^{+}/Na^{+}			Ca^{++}/Na^{+}		
NaCl	Zander level			Zander level		
	0%	10%	30%	0%	10%	30%
Control	0.63	1.13	1.98	5.11	10.91	13.54
0.5 M x 1	0.11	0.25	0.38	0.77	1.80	3.55
0.5 M x 2	0.09	0.21	0.31	0.59	1.49	2.80
0.5 M x 3	0.07	0.15	0.29	0.46	1.03	2.50
1.0 M x 1	0.09	0.23	0.39	0.55	1.44	2.95
1.0 M x 2	0.08	0.17	0.31	0.45	1.04	2.33
1.0 M x 3	0.07	0.13	0.22	0.38	0.78	1.63

5.4 Discussion

The results in Table 5.2 for the chemical and physical properties of Zander show that it is suitable to support plant growth. Zander has a pH range between 6.8 and 6.9, which is favourable for plant growth due to good nutrient availability for plants in this range. Zander is non-saline and has an electrical conductivity of 1.04-1.08 dS m^{-1}, and high moisture content, with high water holding capacity and this may help to decrease the concentration of NaCl in the medium and plant. Organic matter content affects growth and yield by directly supplying nutrients (Darwish, Persaud,, and Martens 1995). Cation exchange characteristics play an important role in soils by determining the retention of any soluble fertilizer. Brady and Weil (1999) state that without cation exchange, the soil is not able to retain sufficient cation nutrients to support natural or introduced vegetation, especially following such events as cultivation or fire. They add that cation exchange and photosynthesis are fundamental life-supporting processes. Genon and Dufey (1991) suggest that the cation exchange capacity (CEC) is one of the most important soil chemical properties with respect to mineral nutrient retention and bioavailability the major cation nutrients (Ca^{++} Mg^{++} K^{+} and Na^{+}). Zander tends to have high cation exchange capacities. Total nitrogen content is 1.88%, which is regarded as good for plant growth. Phosphorus is also important for cell division and enlargement, (Salisbury and Ross 1985).

Salinity disturbs the mineral-nutrient relations in plants through its effect on nutrient availability, transport and partitioning in plants. Additionally, salinity stress also induces ion deficiency or imbalance due to the competition of nutrients such as K^{+}, Ca^{++}, and Mg^{++}, P and Fe with the toxic ions Na^{+} and Cl^{-}. Mineral nutrients play a vital role in

determining plant resistance to salinity. Because salinity affects plant growth through a water deficit, K^+ is equally important to maintain the turgor pressure of the plant under either stress.

The concentration of ions such as calcium, potassium, sodium, magnesium and phosphorus in the plants of *A. saligna* and medium in the greenhouse and the field in Libya was analysed by atomic emission spectroscopy. Control plants died after the second application of salt with 1.0 M NaCl in the field and the third with 0.1 M NaCl in the greenhouse. Consequently, there were no results for these two treatments.

The magnitudes of change in physical and chemical properties of the medium were found to be dependent on Zander application levels (10% and 30%). The results showed that the soil properties were affected by Zander applied to the soil. Furthermore, it became rich in nutrients and had a high water holding capacity, providing favourable conditions for root growth. Zander organic matter led to a change in soil texture from sandy to clayey. Soil texture is the dominant factor in organic matter breakdown in ecosystems (Gregory, Murray, and Schlesinger 2001, Zhao, Zhang, and Zhao 2003). Zander application to soil or sand enables *A. saligna* to grow and to withstand irrigation with NaCl.

The concentration of element uptake by *A. saligna* plants was higher in plants grown in 30% of Zander than in the sand and soil alone. The partially decomposed residue is called effective humus which in previous studies represented about 40% of the total weight (Zander Corporation 2007). The effect of Zander is determined by its effect on growth, as well as on its ionic concentration in the plants. The high ionic concentration in the plants is responsible for most of the biochemical changes which affect the cellular metabolic level, and consequently the growth and development of the plant. Seedling

survival showed that salinity and Zander had significant effects on plant survival ($p < 0.001$). Frequency and concentration of saline water application to plants grown in Zander amended medium had a significant influence on seedling survival of *A. saligna.* The main effect of frequency of irrigation with 0.5 M NaCl was not significant but was highly significant with 1.0 M irrigation. There was interaction between these factors whether with 0.5 M or 1.0 M NaCl irrigation. When plants were grown in pots with 30% Zander and irrigated with 0.5 M saline solution more plants survived than with any other treatment even without salt. Irrigation with 1.0 M NaCl three times still gave survival with 30% Zander greater than with 0% or 10% Zander.

The application of Zander in the low salinity treatment was highly effective compared with the control in soil or sand alone, whereas the high salinity treatment significantly impaired fresh weight of the plants. The application of 30% Zander not only enhanced fresh weight of shoot and root 3-fold compared with the control, but also the salinized one (1.0 M NaCl once) by about 4-fold. Shoot dry matter significantly decreased with increasing salinity. No significant differences in shoot dry matter were recorded when plants were grown without salt applications and with one application of 50 ml NaCl 0.5 M. The highest shoot dry weight was obtained with un-salinized plants grown in the medium containing 30% Zander or 10% Zander. The lowest shoot dry weight was obtained with three applications of 50 ml NaCl at 0.5 M or 1.0M with plants grown in a medium contained 0% Zander or 10% Zander. The decrease in shoot dry matter with salinity of plants grown in medium with no Zander (Control) or low level of Zander might be due to limited supply of metabolites to young growing tissues (Mass and Nieman, 1978) or interference of NaCl with the production of proteins or damage to enzyme proteins exposed to low water potential (Weimberg 1987). On the other hand, results also revealed that there were differences in shoot dry weight owing to the

interaction between frequency, and amount of NaCl concentrations and Zander level. The highest dry weight obtained was detected in the plants grown in a 30% Zander medium, which received 0.5 M NaCl or 1.0 M NaCl. The positive effect of Zander on plant dry weight even under salinization could be ascribed to the presence of sufficient nutrient elements, since the higher the Zander level, the higher the dry weight obtained. In addition, retention of water by the aid of Zander may be helpful in minimizing the hazardous impact of salinity. High salinity caused a reduction in the moisture content of plants and eventually their growth. Glenn (1987) reported that water content of 19 grasses declined with an increase in salinity. Moisture content in *A. saligna* increased with the first application of salt and progressively decreased to the lowest value with three applications of salt at each concentration in the greenhouse and in the field in Libya. This decrease in water content by salinity could be attributed to low ion accumulation in the shoot tissue and to disrupted osmotic balance by reducing the tissue water (Gulzar, Ajmal Khan, and Ungar 2005). On other hand, the concentration of Zander significantly affected moisture content of *A. saligna* owing to its protective effects toward water loss. At high concentrations of salt, the external osmotic potential may be depressed below that of the cell water potential resulting in osmotic desiccation.

The first thing to emphasize in Table 5.3 and 5.19 is the progressive increase in sodium concentration of plants, which was expected due to application of saline solution to the growth medium. This increase in sodium concentration was accompanied by a progressive decrease of potassium concentration. Epstein (1961) showed that there is an antagonistic relationship between K^+ and Na^+ uptake. This antagonism may be due to the direct competition between K^+ and Na^+ at a site of ion uptake in the plasma lemma, of feeder root cells. Sodium may also enhance the efflux of K^+ into the growth medium,

because of disturbance in membrane integrity. The increase in sodium concentration due to application of saline solution was higher with higher amount of Zander.

Substantial differences in Na^+ and K^+ accumulation between salt-resistant species may be due to differences in ion selective transport capacity at root level (Wang *et al*. 2002). Glenn (1987) studied the effect of salinity on the growth of 14 grasses and measured ash and cations. He reported that in response to salt stress, Na^+ increased in shoots, K^+ was decreased and water content decreased. Glenn suggested that grasses maintain osmotic balance by water loss rather than sodium uptake. Zander affected calcium concentrations since there was a great difference in Ca^{++} uptake by plants grown in a medium without Zander than with 10% or 30% of Zander. The average calcium concentrations of plants grown in 10% or 30% Zander were approximately 2 fold that of 0% Zander. The ameliorative effect of Ca^{++} on Na^+ toxicity has been reported (Epstein 1961). Viets (1944) reported that a certain level of calcium is required for maximal uptake of nutrient ions. Calcium acts presumably by maintaining the membrane with its proteins and lipids in a proper physico-chemical state (Rengasamy 1987). There are several reports suggesting a close connection between membrane integrity and calcium, and the consequent effects on ion transport. Supplemental Ca^{++} can affect the length of the growth zones of salt-stressed plants. In sorghum leaves, the length of the growth zone is shortened by 100 mM NaCl salinity. If the Ca^{++} concentration of the nutrient is increased from 1 to 10 mM, then shortening of the growth zone by salinity is prevented. Bernstein, Lauchi, and Silk (1993) found that the addition of Ca^{++} (10 mol m^{-3}) to the saline medium (200 mol m^{-3}) offset the reduction in cotton seedling growth caused by NaCl, by maintaining K^+/Na^+ selectivity and adequate Ca^{++} status in the root. There are several reports supporting the involvement of Ca^{++} signalling in salt tolerance: Ca-

binding protein is induced in salt-stressed *Arabidopsis* (Jang *et al.* 1998) and shows a substantial increase after salinization in mRNA levels of a Ca-ATPase in tomato (Wimmers, Ewing, and Bennett 1992). The experiments also revealed that salinity increased Fe^{++} content in plant tissues. Hassan and Ali (1970) reported that Fe^{++} ion concentration decreased in shoots of barley and corn under salinization.

Zander affected phosphorus concentration when added to the medium and great differences were shown between 0%, 10% and 30% Zander. Uptake of P by plants grown in a medium with 30% of Zander was three times higher than that of plants grown in a medium without Zander and twice that with 10% Zander. In most cases, salinity decreases the concentration of P in plant tissue (Sharpley, Meisinger, and Suarez 1992).

The Ca^{++} content in soil samples varied by salinity and by Zander concentration. A slight variation in Ca^{++} concentrations was observed by salinity when it was compared with variations due to Zander concentration. Frequency of irrigation with 0.5 M NaCl or 1.M NaCl may affect the Ca^{++} status of the soil due to changes in solubility and exchange reactions, and the consequent loss of ions of calcium by leaching and erosion. In non-saline sodic soils, increase in exchangeable sodium is balanced by a decrease in exchangeable Ca^{++} and Mg^{++}, leading to Ca^{++} and/or Mg^{++} deficiencies in plants when these ions are deficient in soil solutions (Rengasamy *et al*. 1984).

Though Ca^{++} concentrations in saline-sodic soils have been reported to be adequate, Ca^{++} level is very low in soils where soluble calcium is absent (Rengasamy 1984). Zander increased Ca^{++} concentration in soil. The availability of micronutrients might be reduced in saline soils as a consequence of pH increase. Maintenance of adequate levels

of K^+ is essential for plant survival in saline habitats. Under saline-sodic or sodic conditions, high levels of external Na^+ interfere with K^+ acquisition by the shoot. Results from solution culture experiments show that the deleterious effects associated with reduced uptake and translocation of K^+ in plants grown in a high Na^+ can frequently be alleviated by the addition of K^+ to the substrate (Grattan and Maas 1985).

The selectivity of the root system for K^+ over Na^+ (Grattan and Maas 1985) together with adequate levels of K^+ provided by Zander, might improve K^+ uptake by plants.

P concentration clearly declined with salinity in soil without Zander. However, P concentration increased as salinity increased in soils with 10% of Zander and 30% Zander. Phosphate availability is reduced in plants with irrigation with saline water because of ionic strength effects that reduce the activity of phosphate but also because phosphate concentration in soil solution is tightly controlled by sorption process and by the low-solubility of Ca-P minerals. Therefore, it is understandable that phosphate concentrations in field-grown agronomic crops decreased as salinity increased. As the increased Na^+ content disturbs the nutritional balance and upsets the osmotic regulation in the plant tissues an antagonistic relation may happen between Na^+ and K^+.

The provision of Ca^{++} from Zander in the root media could prevent the accumulation of toxic Na^+ ions in the plants. The uptake of Na^+ by plants increased progressively with the increased concentration of saline water irrigation and frequency of application whereas uptake of Ca^{++} and P generally decreased and that of Mg^{++} and K^+ were not affected. The values of K^+/ Na^+, Ca^{++}/ Na^+ ratios in the plants increased with increasing the saline water irrigation and Zander level. The relatively low uptake of Ca^{++} due to the

high concentration of Na^+ in the soil solution appeared to increase the adverse effect of sodium on the seedlings.

Acacia saligna responded positively to Zander amendment especially in the low salinity treatment (0.5 M NaCl). Zander treatment at 30% produced taller plants and more plant biomass than 10% and 0% Zander. Zander treatment maintained higher soil water levels than the control, thus confirming that its application enhanced the water holding capacity of the soil. The applications of Zander to sand in greenhouse and to soil in the field in Tripoli Libya increased water holding capacity of soil. Post-harvest soil analysis showed high concentrations of Ca^{++}, Mg^{++}, Na^+, K^+ Fe^{++} and P.

The improvement in growth may also be related to the essential nutrients contained in Zander, water retention and plant nutrients following Zander application. Zander application at 30% increased Ca^{++} concentration in salt stressed plants. The provision of Ca^{++} from Zander to the *A. saligna* in the root media would prevent an accumulation of toxic Na^+ ion in plants. The greater Ca^{++} in Zander may be useful to plants in the saline environments due to ionic interactions in the soil from reduced toxic effect of Na^+.

Hypothesis: Zander improves plant survival and growth in the absence of salinity and ameliorates the effect of salinity by increasing water holding capacity and preventing drought effects.

5.5 Conclusions

Zander supported *A. saligna* growth under saline conditions and ameliorated some of the adverse effects of salt on plants. *A. saligna* grown in 10% or 30% Zander medium mix were able to survive repeated applications of saline water irrigation with 0.5 M NaCl, and experienced only a small reduction in growth with 1.0 M NaCl irrigation while plants grown in sand or soil alone died after the first application of 1.0 M NaCl.

Zander organic amendment increases the nutrient content of the soil. In the absence of NaCl stress the concentration of minerals in plants grown with Zander was greater than those in both sand and soil alone (0% Zander). At all levels of salinity stress applied, mineral concentration of plants was higher with 10% or 30% Zander than with no Zander (0% Zander). Zander increased K^+ concentration and it has been reported that this might serve as an indicator of crop salt tolerance; Zander also provided high amounts of Ca^{++} whose ameliorative effect on Na^+ toxicity has been reported. However, plants grown in Zander mix were also found to contain much higher levels of Na^+ than control plants when irrigated with NaCl.

The moisture content of plants grown in Zander mix was considerably higher than that of control plants. Zander has high moisture content and high water retention capacity which is required for sustained growth of *A. saligna* in saline irrigation. Addition of the Zander organic amendment improved plant growth and also improved soil condition by changing physical and chemical properties, thus enabling growth under NaCl irrigation.

The growth of *A. saligna* in Zander medium not only provides biomass to be used as forage but also ameliorates soil conditions.

Chapter 6

Effect of Zander and additional irrigation on mineral uptake and growth of *Acacia saligna* under saline conditions

6.1 Introduction

Plant growth and survival in semi arid ecosystems is strongly limited by water supply. The already scarce availability of water might further decrease in many areas as a consequence of desertification, which is particularly acute in semi-arid regions (Schlesinger *et al*. 1990). Any factor increasing water availability is likely to increase plant production in these regions. The available water of soil or water holding capacity expresses the amount of water that can be stored in soil for plants during periods without rain and irrigation. This is a key criterion for selection of suitable plants. According to Russell (1973), soil conditioners are generally materials which, when added to soil, improve the physical and chemical properties and make it more favourable for crop production. Zander applied to the soil of Libya as a soil conditioner produced an increase in survival and growth of *A. saligna* (Chapter 5) and it is likely that this is partially due to a change in aggregation and physical properties of soil. Saker and Warid (1977) conducted an experiment to study the effect of addition of the soil conditioner 'Agrosil' on growth and yield of peas, and obtained an increase in yield due to 'Agrosil' application. The physical properties of soil that are improved by amendments include soil structure, porosity and water-holding capacity. Poor soil physical characteristics directly constrain root growth and these may be considered as the biggest problem in agriculture. Organic soil amendments are currently the most common solution for improving the physical characteristics of soils. Tester (1990) found that compost

significantly increased soil organic matter, leading to a direct increase in water holding capacity. This improved soil aggregation, through its effects on soil water content, temperature, aeration and mechanical impedance which influenced root development and seedling emergence (Ferreras *et al.* 2006). In Chapter 5, Zander was shown to have a beneficial effect on the growth and survival of *A. saligna* seedlings, especially when they are exposed to saline irrigation. At least part of the adverse effect of salinity has been ascribed to an osmotic effect imposing a drought stress on plants. The objective of this experiment was to investigate whether additional irrigation of soil with distilled water, when plants were grown without Zander could alleviate the effects of salinity in the same way that incorporation of Zander in the soil does. This was intended to indicate whether the beneficial effect of Zander could be accounted for, at least in part, by the effect it has on soil of increasing water holding capacity and maintaining a water supply to plants during periods of saline irrigation.

6.2 Materials and methods

The experiment was conducted in the Experimental Station of the Faculty of Agriculture, Sidi El Mesri, Tripoli, Libya, during May to July 2009. Environmental data are given in Appendix 1. Seeds of *A. saligna* were sown in pots with a minimum diameter of 130 mm. The bottom of the pots contained a piece of filter paper cut to size to prevent the soil falling out when dry. Seeds were soaked by using 100 ml of boiling water poured onto 80 seeds for each treatment and left to cool; boiling water was applied three times for 30 min as in Chapter 3. The design employed in this experiment was a Randomized Block Design (RBD) with four replicates of each treatment. The soil

material used in this study was taken from a surface horizon of Sidi El Mesri soil. Table 6.1 shows some of characteristics of the soil used.

Table 6.1: Soil physical and chemical characteristics before planting

pH	EC (dS m^{-1})	Sand (%)	Silt (%)	Clay (%)	Soil texture
7.8	5.0	88	9.6	2.4	Sandy loam

After collecting the soil it was air dried and sieved through a 2 mm mesh sieve. Zander was added to soil at three different rates 0%, 10% and 30% by weight. Concentrations of salt irrigation (0.0 M, 1.0 M NaCl) were also investigated in this study. After the addition of Zander, each sample was mixed until homogeneous and placed in 13 cm diameter pots. Pots with no Zander were filled only with soil. Soil and Zander were mixed together by weight at 10% Zander + 90% soil and 30% Zander + 70% soil.

6.2.1 Water holding capacity

A Whatman No. 2 filter paper was placed in the bottom of a plastic pot and the mass of pot and filter paper was determined. The pot was gently filled with dry soil or soil with Zander and the weight of the filter paper and dry samples was determined. The pot was placed in a shallow pan of water allowing the bottom of the pot to become wet. All the pots were irrigated to field capacity; the pot was then removed from the pan and placed in a humid enclosure until water drainage was complete. After that the mass of the pot, filter paper and saturated soil sample was determined.

Mass of dry soil = (Mass of pot + filter paper + dry soil) **-** (Mass of pot + filter paper)

Mass of the saturated soil = (Mass of pot+ filter paper + saturated soil) **-** (Mass of pot + filter paper)

Mass of water contained in the saturated soil = Mass of the saturated soil - Mass of dry soil.

Field capacity of soil and soil with 10% or 30% Zander was determined. The water holding capacity of 30% Zander was 160 ml with 150 g Zander + 350 g dry soil, with 10% Zander water holding capacity was 85 ml in 50 g Zander + 450 g dry soil and with 0% Zander it was 48.5 ml water with 500 g soil alone. The extra water required to supply the same water as the holding capacity with 30% Zander mix was added to 0% or 10% Zander. Pots with 0% Zander had 111.5 ml more distilled water added and those with 10% Zander received 75 ml extra distilled water in order to be equivalent to the water availability with 30% Zander.

6.2.2 Irrigation schedule

In the first weeks all pots (48 pots) were irrigated with distilled water to field capacity and any leachate was retained in the saucers. Pots with 0% Zander had 48.5 ml +111.5 ml more distilled water added and those with 10% Zander received 85 ml + 75 ml extra distilled water in order to prove equivalent to the water holding capacity as 30% Zander (160 ml). Extra water from 0% and 10 % Zander leachate was retained from the saucers to pots every day. During the third week all the plants were watered with 50 ml of 1.0 M NaCl solution while the control received 50 ml distilled water. After the fourth week all plants were irrigated to field capacity for each treatment, with or without extra water, and at the end of seven weeks all plants were harvested. This regime of application is summarized all treatments in Table 6.2.

Table 6.2 Irrigation applied on each occasion to pots (DW= Distled water ml)

Zander	Treatment				
	Time	Con	1.0 M NaCl	Con + water	Con + 1.0 M NaCl + water
0%	WK1	45.5 D.W.	45.5 D.W.	160 D.W.	160 D.W.
	WK2	45.5 D.W.	45.5 D.W.	160 D.W.	160 D.W.
	WK3	50 D.W.	50 NaCl	50 D.W.	50 NaCl
	WK4	45.5 D.W.	45.5 D.W.	160 D.W.	160 D.W.
	WK5	45.5 D.W.	45.5 D.W.	160 D.W.	160 D.W.
	WK6	45.5 D.W.	45.5 D.W.	160 D.W.	160 D.W.
	WK7	Harvest	Harvest	Harvest	Harvest
10%	WK1	85 D.W.	85 D.W.	160 D.W.	160 D.W.
	WK2	85 D.W.	85 D.W.	160 D.W.	160 D.W.
	WK3	50 D.W.	50 NaCl	50 D.W	50 NaCl
	WK4	85 D.W.	85 D.W.	160 D.W.	160 D.W.
	WK5	85 D.W	85 D.W	160 D.W	160 D.W
	WK6	85 D.W.	85 D.W.	160 D.W.	160 D.W.
	WK7	Harvest	Harvest	Harvest	Harvest
30%	WK1	160 D.W.	160 D.W.	160 D.W.	160 D.W.
	WK2	160 D.W.	160 D.W.	160 D.W.	160 D.W.
	WK3	50 D.W.	50 NaCl	50 D.W.	50 NaCl
	WK4	160 D.W.	160 D.W.	160 D.W.	160 D.W.
	WK5	160 D.W.	160 D.W.	160 D.W.	160 D.W.
	WK6	160 D.W.	160 D.W.	160 D.W.	160 D.W.
	WK7	Harvest	Harvest	Harvest	Harvest

WK= Week

6.2.3 Determination of soil moisture content

Three pots in which there were no seedlings were used to measure the moisture content of the soil and soil mix with Zander. Zander was added to the soil sample in the three different levels by weight, 30% Zander was 150 g Zander + 350 g dry soil, with 10% Zander it was 50 g Zander + 450 g dry soil and with 0% Zander it was 500 g dry soil alone. The pots were irrigated to field capacity after irrigation 50 g of fresh soil and soil + Zander was weighed and left to dry completely in the green house for a week, then the soil samples were weighed again to find out the moisture of the soils and soil + Zander.

Moisture content was measured using the equation (Equation 6.1)

$$\text{Moisture of soil} = \frac{W1 - W2}{W1} \times 100\%$$

6.2.4 Bulk density

Bulk density was determined by measuring the mass of dry soil with Zander at 0%, 10% and 30%. Three replicates of each sample of fresh weight soil or soil mixed with Zander about 10 g were placed in the foil trays, weighed and dried in the oven (Oven 300 plus series) at 80^{o}C for 28 h, allowed to cool and reweighed. Soil bulk density was determined by measuring the mass of dry soil per unit of volume using a measuring cylinder (g cm^{-3}), (Blake and Hartge 1986).

$$\text{Bulk density} = \frac{\text{Mass of the dry soil (g)}}{\text{Total volume of soil (cm}^3\text{)}}$$

6.2.5 Sample preparation and analysis

Soil and Zander mixture in each pot was air dried and sieved through a 2 mm sieve. From each pot 100 g of the soil sample was put in a small bag and labelled for subsequent analysis. The methods used in Chapter 4 to analyse soil and plants were employed here for analysis of samples in the laboratory, including pH, water holding capacity, EC and major nutreants.

6.2.6 Fresh and dry weights

Surviving of all the plants from each pot were harvested in July 2009. The plants were harvested carefully and the roots washed free from debris with running water. Plants were carefully blotted dry with tissue and fresh weight was determined immediately. Plants were counted and plant height was recorded and leaf area determined with a Li-3100 leaf area mater (Li-COR. Inc., Lincoln, NE, USA). The plants were divided into root and shoot using scissors; and placed into plastic bags after harvesting to keep them fresh until they could be taken to the laboratory. Plants were removed from plastic bags and placed in an air circulation oven and dried at 80°C for 24 h, then allowed to cool to room temperature in desiccators and reweighed.

6.2.7 Statistical Analysis

The significance of differences between means was tested by three-way analysis of variance using Minitab 15 Computer Package. The mean separation followed by the calculation of a least significant difference for all paired comparisons was undertaken using Tukey's test at $p \leq 0.05$. Final % survival data was arcsin transformed before analysis.

6.3 Results

6.3.1 Soil physical properties

Table 6.3 shows that addition of Zander to the soil improved the soil physical properties and characteristics such as pH, electrical conductivity, organic matter and total nitrogen. The highest concentration of total nitrogen in the soil was with 30% Zander and the lowest with 0% Zander. When Zander was added to soil the organic matter increased from negligible levels in soil alone to 36% with soil and 30% Zander mixed (Table 6.3). The pH decreased with increased Zander level, Zander lowered the pH from 7.8 with 0% to 7.5 at 10% Zander and 6.5 at 30% Zander. Addition of Zander to the soil increased electrical conductivity with values of 8.8, 6.3 and 5.5 (dS m^{-1}) at 25 ºC with 30%, 10% and 0% Zander respectively (Table 6.3) moisture content was higher with 30% and 10% Zander than with 0% Zander (Table 6.3). Soil texture also changed with addition of Zander to soil, from sand to sand and clay with 10% Zander, and to clay with 30% Zander (Table 6.4).

Table 6.3 Soil physical properties with addition of different Zander concentrations before plant growth

Zander level	pH	EC (dS m^{-1})	OM (%)	N (%)	MC (%)	WHC (ml 500 g^{-1})	B.D (gcm^{-3})
0%	7.8	5.5	0.32	0.02	14.00	45.5	1.63
10%	7.5	6.3	12.00	0.55	29.00	85.0	1.24
30%	6.5	8.8	36.00	1.22	32.00	160.0	1.08

Table 6.4 Soil particle size distribution

Zander, level	Clay (%)	Slit (%)	Sand (%)	Soil texture
0%	2.40	9.60	88.00	Sandy
10%	51.82	2.20	45.98	Sandy clay
30%	62.40	1.95	35.65	Clay

6.3.2 Seedling survival

Figure 6.1 shows that in the absence of salinity stress and no extra water, Zander at 10% significantly increased survival of plants and this was further significantly increased with the 30% Zander. The addition of extra water to pots with 10% Zander significantly increased survival to the same level as with 30% Zander and no extra water. Addition of extra water to pots with 0% Zander significantly increase plants survival compared with control plants but not to same level as 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased seedling survival with all levels of Zander compared with no stress plants. With saline stress, Zander at 10% significantly increased survival of plants and this was further significantly increased with 30% Zander. With salinity and addition of extra water to pots with 10% Zander survival was not significantly different from treatments with 30% Zander and no extra water. Addition of extra water to pots with 0% Zander significantly increased survival of plants but not to the same level as with 30% Zander and no extra water.

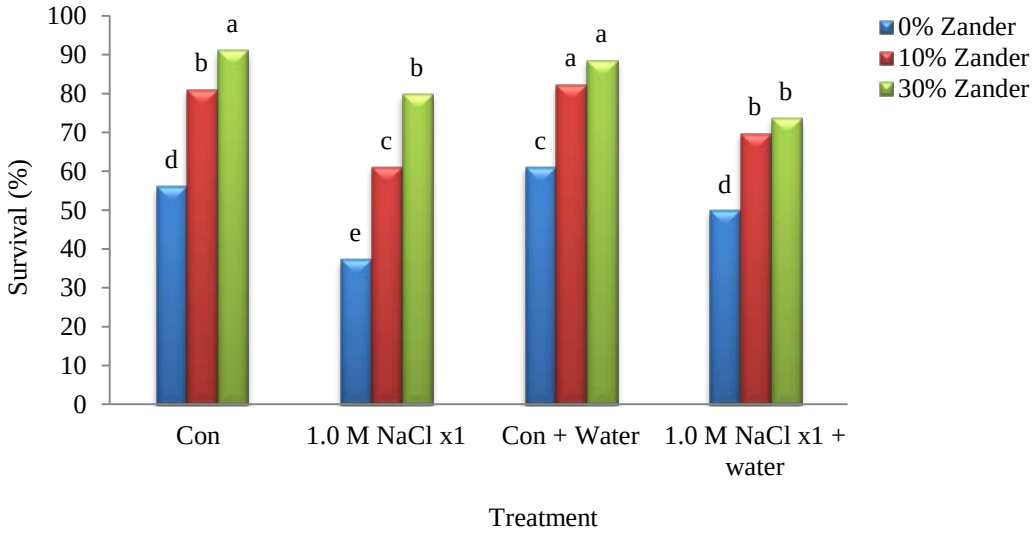


Figure 6.1: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on the survival of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

6.3.3 Shoot height, leaf number and leaf area

Figure 6.2 shows that shoot height in the absence of salinity stress was significantly increased by addition of 10% Zander to soil and this was further significantly increased with 30% Zander. Addition of extra water to pots with 0% or 10% Zander increased shoot height significantly compared with controls but not to the same level as with 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased shoot height of plants with all levels of Zander compared with controls. With salinity stress, addition of extra water to pots with 0% or 10% Zander significantly increased shoot height but not to the same level as with 30% Zander and no extra water. Addition of extra water significantly improved shoot height but not as good as 30% Zander and no extra water. Thus addition of extra water partly but not completely replicated the beneficial effect of Zander on shoot height.

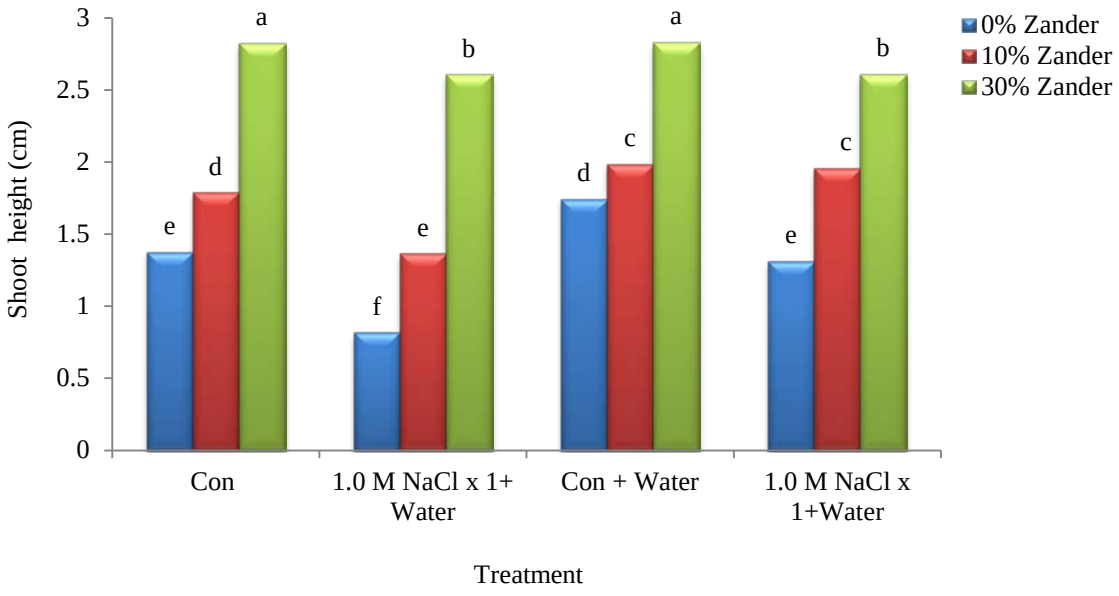


Figure 6.2: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on the shoot height of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 6.3 shows the leaf number of plants grown with Zander with or without salinity stress. In the absence of salinity Zander at 10% significantly increased number of leaves and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 10% Zander increased number of leaves to the same level as with 30% Zander and no extra water. Addition of extra water to pots with 0% Zander increased number of leaves to the same level as with 10% Zander and no extra water but did not increase to the same level as 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased leaf number with 0% Zander but not with 10% or 30% Zander. With salinity stress, addition of extra water to pots with 10% Zander significantly increased number of leaves as the same level as with 30% Zander and no extra water. With salinity stress addition of extra water to pots with 0% Zander also significantly increased number of leaves but not as the same level as with 30% Zander and no extra water.

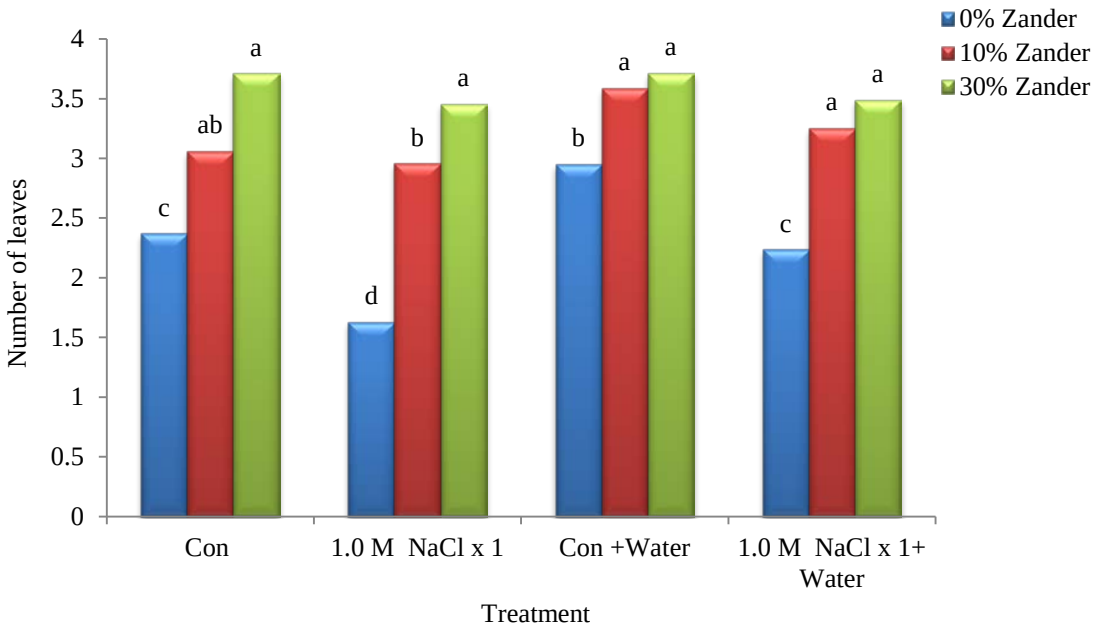


Figure 6.3: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on the number of leaves of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 6.4 shows the leaf area of plants grown with Zander. In the absence of salinity stress Zander at 10% produced no significant increase in leaf area compared with plants with 0% Zander but this was significantly increased with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 0% and 10% Zander did not significantly increase leaf area compared with equivalent controls nor was it increased as to the same level as 30% Zander and no extra water. With saline stress, applied as one application of 1.0 M saline solution, leaf area of plants was significantly decreased for all levels of Zander. With salinity stress addition of extra water to pots with 0% and 10% Zander significantly increased leaf area compared with equivalent treatments without additional water. Moreover, in the case 10% Zander this was not significantly different from 30% Zander and no additional water. Thus, the addition of extra water partly but not completely replicated the beneficial effect of Zander on leaf area.

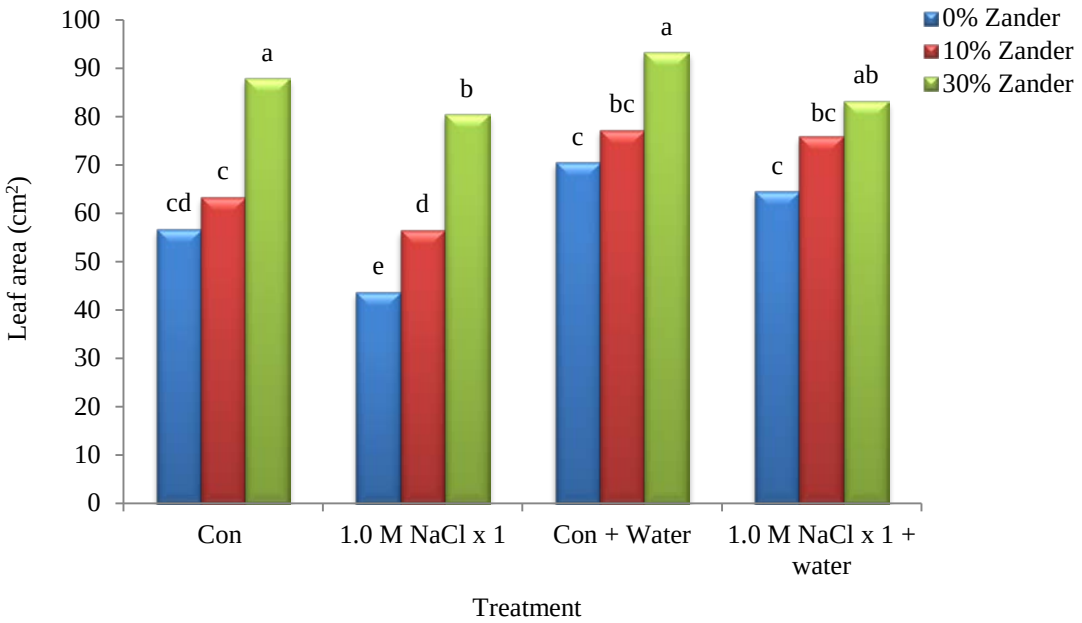


Figure 6.4: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on the leaf area of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

6.3.4 Shoot fresh and dry weight

Figure 6.5 shows that when plants were grown in 10% Zander and in the absence of salinity stress, fresh weight of shoots was significantly increased compared with 0% Zander and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 0% and 10% Zander significantly increased shoot fresh weight but not to the same level as with 30% Zander and no extra water. Saline stress applied as one application of 1.0 M NaCl significantly decreased shoot fresh weight for all levels of Zander. With salinity stress and addition of extra water, pots with 0% and 10% showed significantly increased shoot fresh weight but not to the same level as with 30% Zander and no extra water.

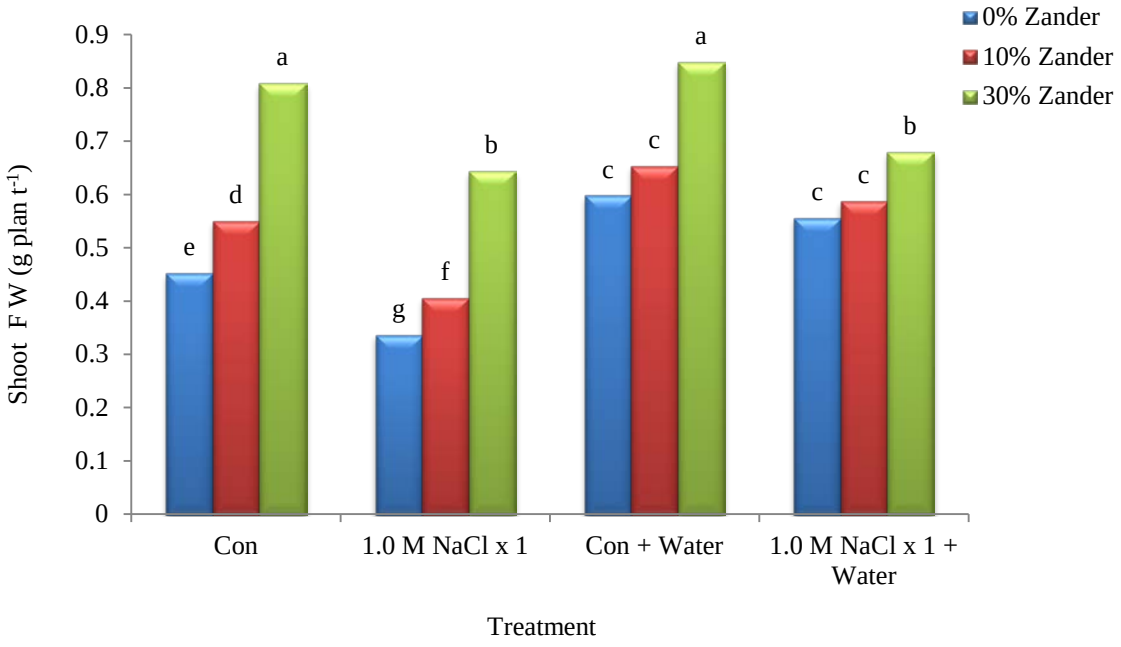


Figure 6.5: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot fresh weight of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 6.6 shows that in the absence of salinity stress, Zander at 10% significantly increased shoot dry weight and this was further significantly increased with 30% Zander compared to 0% Zander. In the absence of salinity stress, addition of extra water to pots with 10% Zander did not significantly increase shoot dry weight and it remained significantly below with 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased shoot dry weight at all levels of Zander. With salinity stress, addition of extra water to pots with 0% Zander significantly increased shoot dry weight but not to the same level as with 30% Zander and no extra water. However, with addition of extra water to pots with 10% Zander shoot dry weight significantly increased to the same level as with 30% Zander and no extra water. Thus, the addition of extra water partly but not completely replicated the beneficial effect of Zander on shoot dry weight.

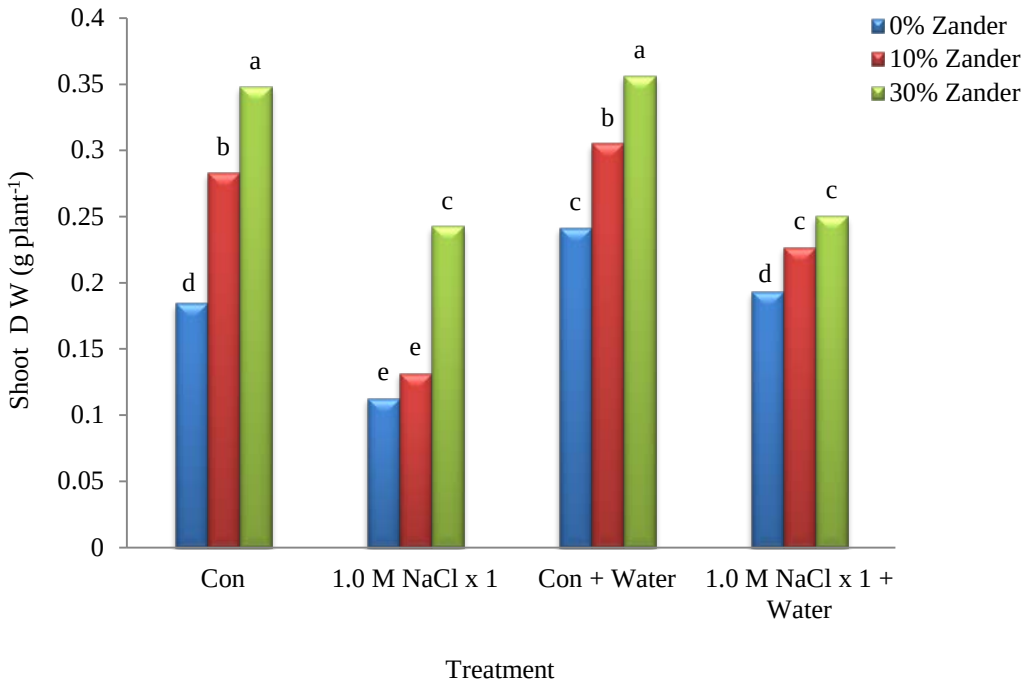


Figure 6.6: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot dry weight of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

6.3.5 Shoot moisture

Figure 6.7 shows that in the absence of salinity stress, addition of Zander at 10% or 30% significantly decreased shoot moisture compared with 0% Zander. In the absence of salinity stress addition of extra water to pots with 0% Zander significantly decreased shoot moisture compared to the equivalent treatments without water. However, there was no significant different between Zander at 10% and 30%. Saline stress, applied as one application of 1.0 M NaCl significantly increased moisture content with 10% or 30% Zander but not with 0% Zander. With salinity stress additional of extra water to pots with salinity stress significantly reduced shoot moisture content and this was significantly below treatments with 30% Zander and no extra water. However, with 0% Zander shoot moisture showed no significant change.

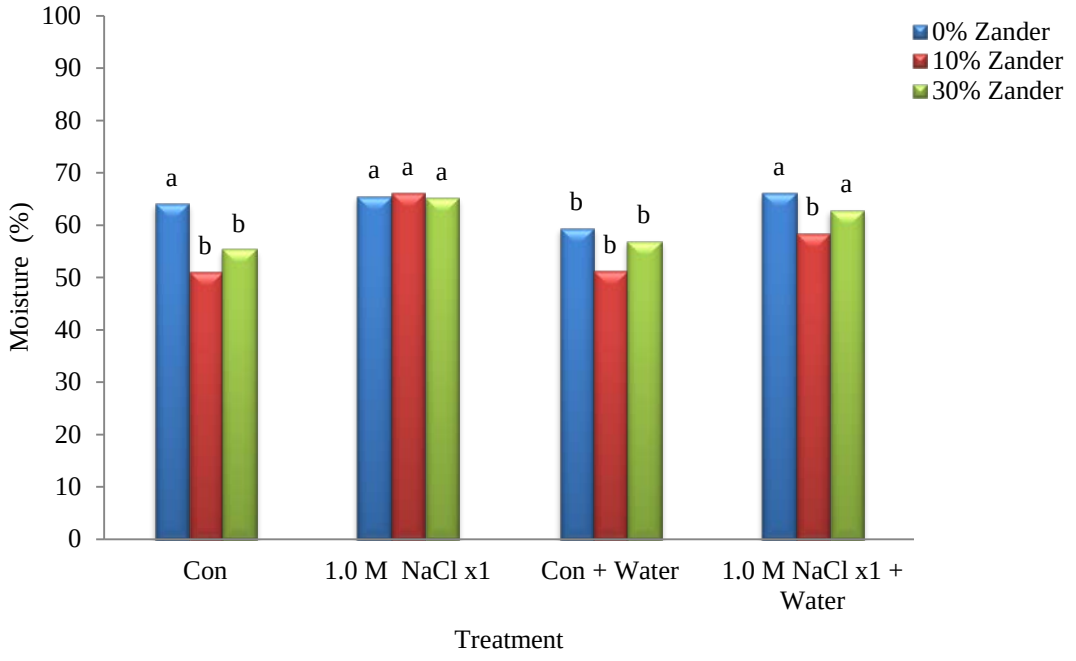


Figure 6.7: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot moisture content of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

6.3.6 Root fresh and dry weight

In the absence of salinity stress, Zander at 10% significantly increased root fresh weight of plants and this was further significantly increased with 30% Zander (Figure 6.8). In the absence of salinity stress, addition of extra water to pots with 10% Zander decreased root fresh weight compared with control. Addition of extra water to pots with 0% Zander significantly increased root fresh weight but not to the as same level as 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased root fresh weight with all levels of Zander. With salinity stress, addition of extra water to pots with 10% Zander did not significantly increase root fresh weight but not to the same level as with 30% Zander and no extra water. Addition of extra water to pots with 0% Zander significantly increased root fresh weight but not to as the same level as with 10% Zander and no extra water.

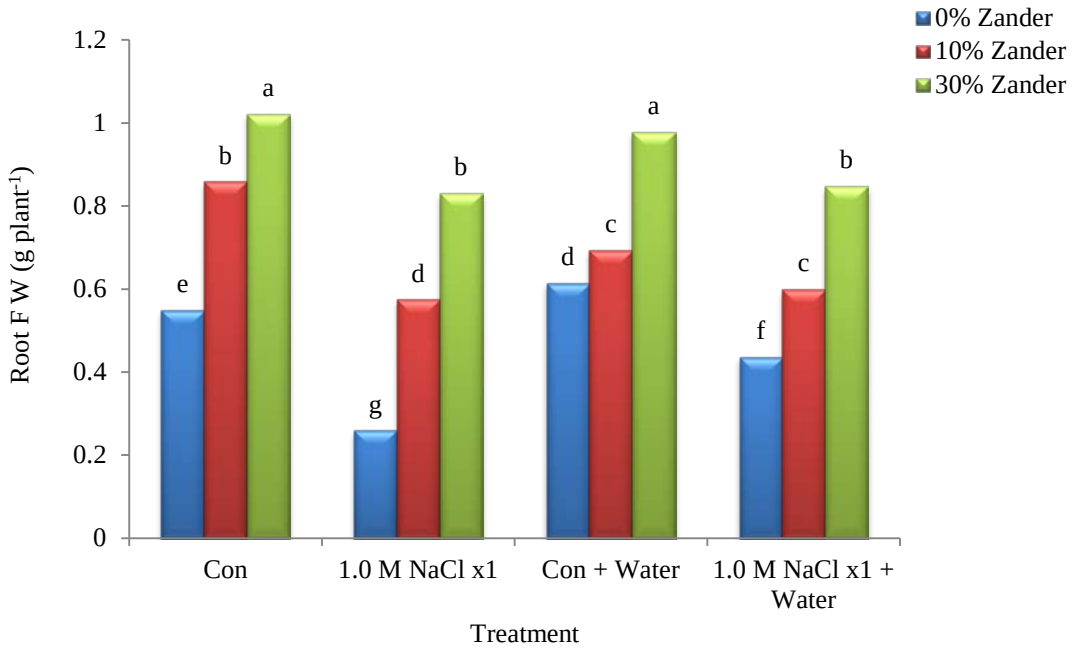


Figure 6.8: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on root fresh weight of *Acacia saligna* grown in medium with or without extra water to pots with three levels of Zander increasing in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 6.9 shows that in the absence of salinity stress. Zander at 10% significantly increased root dry weight and there was a further significant increase with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 10% Zander significantly increased root dry weight to the same levels as with 30% Zander and no extra water. Addition of extra water to pots with 0% Zander significantly increased root dry weight but not the same level as with 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl, significantly decreased root dry weight with all levels of Zander. With salinity stress, addition of extra water to pots with 0% and 10% Zander significantly increase root dry weight but not to the same level as with 30% Zander and no extra water. Thus addition of extra water partly but not complelty replicated the beneficial effect of Zander on root dry weight.

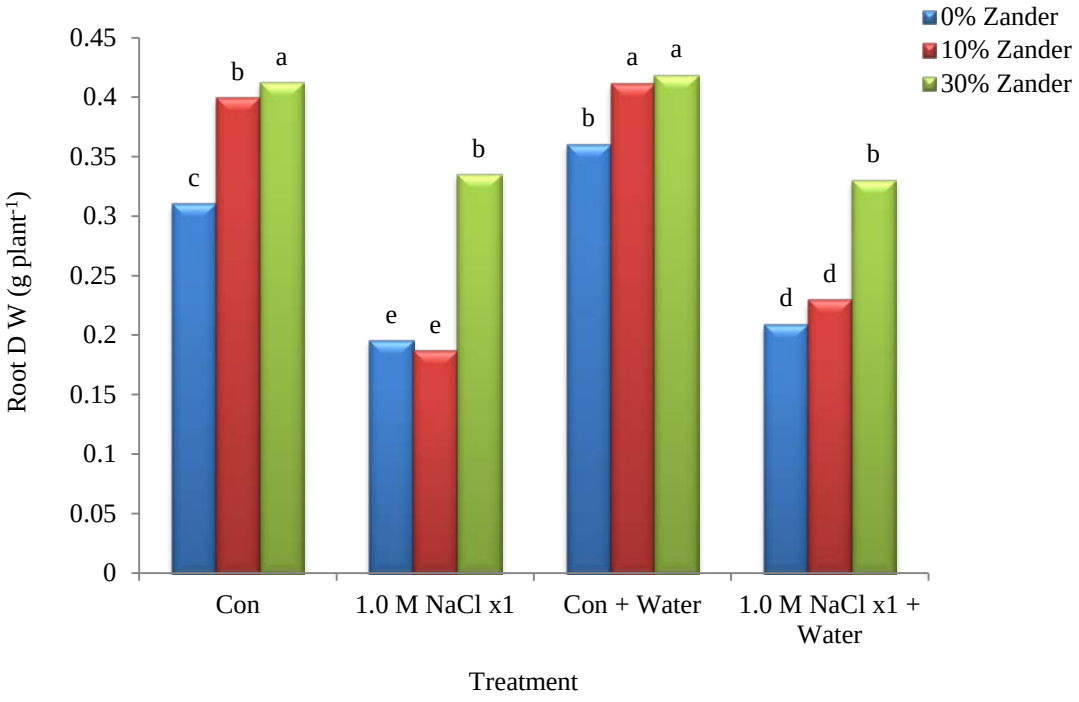


Figure 6.9: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on root dry weight of *Acacia saligna* grown in medium with or without extra water to pots with three different concentrations of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

6.3.7 Root moisture content

Figure 6.10 shows that in the absence of salinity stress, root moisture content in treatments without Zander 0% was significantly lower than with either 10% and 30% Zander although these were not significantly different from each other. The application of saline stress, with or without additional water, had no significant effect on root moisture content.

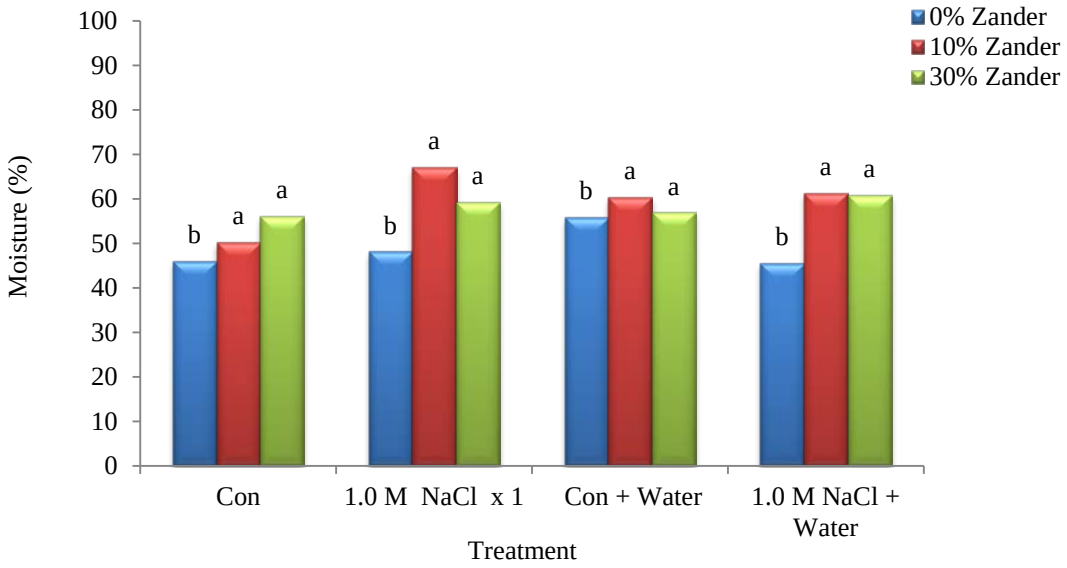


Figure 6.10: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on root moisture content of *Acacia saligna* grown in medium with or without extra water to pots with three different concentrations of Zander in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

The hypothesis was that Zander improves plant survival and growth in the absence of salinity and ameliorates the effect of salinity by increasing water holding capacity and preventing drought effects. Increasing the supply of water to the plants by irrigation improve and increases survival, growth and salinity tolerance even in the absence of Zander However, extra water can improve early survival and growth of *A. saligna* seedlings grown under salinity stress.

Table 6.5 shows the beneficial effect of addition of extra water on survival and plant perimeters of *Acacia saligna* grown in medium with and without saline stress in the field in Libya.

Table 6.5: Does extra irrigation applied account for the beneficial effect of Zander on plant growth

	No salt		With salt	
	0% Zander	10% Zander	0 % Zander	10% Zander
Survival %	+	++	+	++
Stem height	+	+	+	+
Leaf number	+	++	+	++
Leaf area	-	-	+	++
Shoot FW	+	+	+	+
Shoot DW	+	+	+	++
Shoot moisture	-	-	-	-
Root FW	+	-	+	-
Root DW	+	++	+	+
Root moisture	-	-	-	-

+ = Significant increase by addition of H_2O

+ + = Significant increase to at least to the same level as with 30% Zander and no extra water.

6.4 Mineral content in plants

Table 6.6 shows that in the medium without salinity stress. Zander at 10% did not significantly increase Na^+ concentration of plants but addition of Zander at 30% did produce a significant increase in Na^+ concentration. In the absence of salinity stress, addition of extra water to pots with 0% Zander significantly decreased Na^+ concentration. Saline stress, applied as one application of 1.0 M NaCl significantly increased Na^+ concentration in plants with 0%, 10% and 30% Zander compared with the control but there was no significant difference between 10% and 30% Zander. With salinity stress, addition of extra water to pots with 0% and 10% Zander did not significantly decrease Na^+ concentration of plants compared to pots without extra water.

Table 6.6: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Na^+ content of *Acacia saligna* grown in medium with extra water to pots with three different concentrations of Zander in the field in Libya

	Na^+ Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	206.5 [d]	285.4 [d]	398.2 [c]
1.0 M NaCl x 1	8594.5 [b]	10061.2 [a]	11234.9 [a]
Con + water	113.7 [e]	209.3 [d]	391.9 [c]
1.0 M x 1 NaCl + water	6699.6 [b]	8984.8 [b]	11235.2 [a]

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 6.7 shows that in the absence of salinity stress, Zander at 10% significantly increased Ca^{++} and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 10% Zander did not increase Ca^{++} concentration in the plants compared with the control and this remained significantly below the level with 30% Zander and no extra water. Addition of extra

water to pots with 0% Zander significantly increased Ca^{++} compared with control but again did not increased Ca^{++} to the same level as 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased Ca^{++} will all levels of Zander. With saline stress, Zander at 10% significantly increased Ca^{++} concentration of plants compared with plants without Zander and this was further significantly increased with 30% Zander. With salinity stress, addition of extra water to pots with 10% Zander significantly increased Ca^{++} concentration in plants such that this was significantly different but not as level as with 30% Zander and no extra water. Addition of extra water to pots with 0% Zander significantly increased Ca^{++} concentration but not to the same level as with 30 % Zander and no extra water.

Table 6.7: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Ca^{++} content of *Acacia saligna* grown in medium with extra water to pots with three different concentrations of Zander in the field in Libya

	Ca^{++} Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	14080.6 ^{d}	16226.5 ^{b}	19113.9 ^{a}
1.0 M NaCl x 1	9299.6 ^{f}	12330.1 ^{d}	16267.1 ^{b}
Con + water	14895.2 ^{c}	16912.9 ^{b}	19118.3 ^{a}
1.0 M x 1 NaCl + water	11313.1^{e}	14376.9 ^{c}	16261.6 ^{b}

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 6.8 shows K^{+} concentration in *A. saligna* plants. In plants grown in the medium without salinity stress, Zander at 10% did not significantly increase K^{+} in plants but there was a significant increase with 30% Zander. In the absence of salinity stress, addition of extra water to pots did not significantly alter K^{+} concentration for equivalent levels of Zander. Saline stress, applied as one application of 1.0 M NaCl significantly decreased K^{+} with all levels of Zander compared with controls with no saline treatment.

With saline stress, Zander at 10% did not significantly increase K^+ concentration in plants compared to plants grown in the absence of Zander. However, a significant increase was found with 30% Zander. With salinity stress, addition of extra water to pots with 0% and 10% Zander resulted is no significant change in K^+ concentration and this was not significantly different from K^+ levels with 30% Zander and no extra water. Thus, the addition of extra water was unable to replicate the beneficial effect of Zander on K^+ concentration in plants.

Table 6.8: Effect of NaCl irrigation at 50 ml with 1.0 M NaCl on K^+ content of *Acacia saligna* grown in medium with extra water to pots with three different concentrations of Zander in the field in Libya

	K^+ Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	10057.6^{b}	12994.9^{b}	14999.6^{a}
1.0 M NaCl x 1	6280.8^{c}	8917.9^{c}	11442.8^{b}
Con + water	10655.7^{bc}	11373.1^{b}	15358.6^{a}
1.0 M x 1 NaCl + water	9946.5^{bc}	9217.1^{bc}	11599.01^{b}

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 6.9 shows that in the absence of salinity stress. Zander at 10% did not significantly increase Mg^{++} of plants but there was a significant increase with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 10% Zander did not significantly alter Mg^{++} concentration and it remained significantly below the level with 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl produced no significant change in Mg^{++} concentrations with 0% and 10% Zander but it was significantly lowered with 30% Zander.

With salinity stress, addition of extra water result no significant change in Mg^{++} levels between different levels of Zander or between saline stressed plants with and without water for equivalent levels of Zander.

Table 6.9: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Mg^{++} content of *Acacia saligna* grown in medium with extra water to pots with three different concentrations of Zander in the field in Libya

	Mg^{++} Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	2726.2 [b]	3093.9 [b]	3934.7[a]
1.0 M NaCl x 1	2001.1[b]	2233.2[b]	3175.1[b]
Con + water	2826.2[b]	3327.3[b]	3928.1[a]
1.0 M x 1 NaCl + water	2334.3[b]	2699.9 [b]	3141.7[b]

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 6.10 shows that in the absence of salinity stress, Zander at 10% significantly increased P concentration of plants and this further was significantly increased with 30% Zander. In the absence of salinity stress, addition of extra water to pots with 0% and 10% Zander did not significantly change P compared with equivalent controls without water or increased to levels with 30% Zander and no extra water. Saline stress, applied as one application of 1.0 M NaCl significantly decreased P concentration with all levels of Zander. However, with saline stress, Zander at 10% significantly increased P of plants and this was further significantly increased with 30% Zander. With salinity stress and addition of extra water, both 0% and 10% Zander treatment resulted in no significant change in the P of plants compared to treatments without extra water, and this remained significantly below the level with 30% Zander and no extra water.

Table 6.10: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on P content of *Acacia saligna* grown in medium with extra water to pots with three different concentrations of Zander in the field in Libya

	P Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	1076.2 ^{c}	1708.9 ^{b}	2252.5^{a}
1.0 M NaCl x 1	473.5^{d}	851.5^{c}	1560.6^{b}
Con + water	1096.1^{c}	1756.3b^{b}	2281.6 ^{a}
1.0 M x 1 NaCl + water	498.9^{d}	877.8 ^{c}	1528.5 ^{b}

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 6.11 shows that addition of extra water at 10% to the soil increased Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio of the plants without NaCl stress. Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio in the plants grown in the soil alone (0% Zander) was higher than 10% and 30% Zander and decreased substantially in a plants with 10% and 30% Zander. Ca^{++}/Na^{+} ratio in the medium with 30% Zander was greater than with 0% and Zander + extra water and with 10% Zander for all frequencies and concentrations of NaCl.

Table 6.11: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on K^{+}/Na^{+} and Ca^{++}/Na^{+} ratio of *Acacia saligna* grown in medium with extra water to pots with three different concentrations of Zander in the field in Libya

	K^{+}/Na^{+}			Ca^{++}/Na^{+}		
Treatment	Zander level			Zander level		
NaCl	0%	10%	30%	0%	10%	30%
Con	48.70	45.53	37.67	68.19	56.86	48.00
1.0 M NaCl x 1	0.730	0.89	1.019	1.08	1.231	1.45
Con + water	93.71	54.34	39.19	131.00	80.80	48.78
1.0 M NaCl x 1+ water	1.48	1.04	1.03	1.69	1.60	1.45

Table 6.12 shows summary of the beneficial effect of addition of extra water on ion uptake by plant of *Acacia saligna* grown in medium with and without saline stress and effect of extra water to plants in the field in Libya

Table 6.12: Does extra irrigation applied account for the beneficial effect of Zander on ion uptake

	No salt		With salt	
	0% Zander	10% Zander	0 % Zander	10% Zander
Na+	+	-	-	-
Ca^{++}	+	_	+ +	+ +
K^{+}	-	-	+	+
Mg^{++}	-	-	-	-
P	-	-	-	-
Ca^{++}/ Na^{+}	+ +	+ +	+ +	+ +
K^{+}/Na^{+}	+ +	+ +	+ +	+ +

+ = Significant increase by addition of H_2O and Na^{+}

+ + = Significant increase to at least to the same level as with 30% Zander and no extra water

6.5 Discussion

Zander has a high water holding capacity. Soil properties, such as electrical conductivity and soil texture have a major effect on the physical characteristics of soil. Zander changed the soil texture from sand to sandy clay with 10% Zander and to clay with 30% Zander. Zander is a source of organic matter in the soil mixture. Organic matter is able to bind soil particles in a more stable structure. The mixture of soil with Zander resulted in increased water holding capacity compared with the soil alone. These results agree with Tester (1990) who found that compost amendment significantly raised water holding capacity. Ferreras *et al*. (2006) also found that application of different compost to a soil significantly raised water holding capacity. Demeyer, Voundi, and Verloo (2001) found that wood ash, when used as an amendment improved the physical characteristics of soil. Zebarth *et al*. (1999) and Franzluebbers (2002) also found the physical characteristics of soil improved depending on the amount of organic matter used.

Soil texture is an important characteristic of the soil. It decisively influences a number of soil attributes such as soil moisture content, permeability and infiltration rate, which are critical for understanding soil behaviour and management. From the soil texture many conclusions can be drawn. Soil texture governs the moisture and nutrient storage capacity. It provides a measure of permeability and to some extent of water holding capacity (Brady and Weil 1999). Soil was changed to clay by Zander which is rich in nutrient reserves and has a high water holding capacity; this result agree with Zhu and Chen (1994) and Su *et al*.

(2003) who found organic matter increased water holding capacity and improved soil physical properties.

Bulk density is dependent on soil texture and the densities of soil mineral and organic matter as found by Sessitsch *et al.* (2001). Soil structure depends on the association between mineral soil particles (sand, silt and clay) and organic matter. Bulk density reflects the soil ability to function for structural support, water movement from soil and aeration. Zander improved soil structure and provided organic matter which decreases bulk density. A reduction in bulk density was recorded, probably because of high organic matter content in the soil when Zander was added and this agrees with Tester (1990) who found that compost significantly reduced bulk density. This result also agrees with Schjonning, Christensen, and Carstensen (1994) who reported a reduction in bulk density of soil due to application of cattle manure in a long-term integrated nutrient management experiment. By adjusting the extra water to the growing media to same level as with 30% Zander (by adding extra water to 0% or 10% Zander), there was a beneficial effect of Zander on percentage survival and plant growth of *Acacia saligna* grown in medium with extra water applied to pots in the field in Libya.

The hypothesis was that Zander may improve seedling growth and plant survival by increasing the water holding capacity of the soil and regulating the plants available water. The successful establishment of agricultural crops depends on moisture availability and is often restricted by poor soil moisture availability particularly in arid and semi-arid environments. The use of soil conditioners had been suggested to improve moisture content in coarse soils (McGuire, Carrow, and Troll 1978). Tester (1990) found that compost significantly increased soil organic matter, leading to a direct increase in water holding capacity.

The addition of extra water to pots with 10% Zander increased survival to the same level as with 30% Zander and no extra water. Extra water added to pots with 0% Zander also increased plant survival but not to same level as 30% Zander and no extra water. The growth and survival of *A. saligna* amended with 10% Zander and 0% Zander was positively increased with the increase water. This result agrees with Khaleel, Reddy and Overcash (1981) who concluded that the water holding capacity of loamy and clay soil increases with application of compost. Thus water is important to the general health of plants and microbes (Eweis *et al*.1998),

Significant reduction in plant survival occurred with one application of 1.0 M NaCl with all levels of Zander compared with the untreated plants. With salinity and addition of extra water to pots with 10% Zander survival was significantly increased to the same level as with 30% Zander and no extra water. While the amount of extra water available to plants was increased by Zander, the period of availability of this extra water is also important for plants and is determined by the rate of evaporation from the soil. Yield increment due to improvement of soil moisture is expp by Shaaban (2006) who found applying both composted and chicken manure improved yield of okra plants.

Extra water significantly increased the number of leaves and leaf area to the same level as with 30% Zander and no extra water with salinity stress. On the other had addition of extra water to pots with 0% Zander also significantly increased number of leaves and leaf area but not to the same level as with 30% Zander. Extra water had a significant effect on fresh and dry weight with or without salinity stress, and addition of extra water to pots with 0% or 10% Zander increased shoot height but not to the same level as with 30% Zander and no extra water. Extra water applied had significant effect only on shoot

dry weight. Extra water significantly improved shoot dry weight to the same level as with 30% Zander and no extra water, while with or without salinity stress, addition of extra water to pots with 0% or 10% Zander increased shoot fresh weight but not to the same level as 30% Zander. 10% Zander and increased watering, led to increased fresh weight of plants. However, shoot moisture did not significant change. These results agree with Faten (2005) and Yasmeen *et al*. 2009) who reported the beneficial effect of chicken manure on growth and yield of different vegetables due to water holding capacity and available moisture.

With salinity stress, addition of extra water to pots with 10% Zander did not significantly increase root fresh weight. The results indicate that there was a significant increase in root fresh and dry weight with 0% Zander with extra water but not to the same level as with 30% Zander and no extra water. However, in the absence of salinity stress, Zander at 0% significantly increased root fresh weight of plants but not with 10% Zander although these results were removed with saline stress. Root moisture content, with or without salt and addition of extra water, showed no significant change. Brown *et al*. (1987) found that water content increased crop growth rate by an increase in leaf area and also by an increase in radiation use efficiency. The results from this study agree with Brugnoli and Bjorkman (1992) who found that roots were less affected by salinity than shoots. The reduction in water uptake by plants due to salt stress has been investigated. Studies suggest that plant root porosity (expressed as the hydraulic conductivity of the root system) declines significantly with an increase in salinity stress and accordingly there is a reduction in water uptake by plants (Pessarakli 1994).

The macronutrient uptake of *A. saligna* plants was studied with various treatments. In the absence of salinity stress addition of extra water to pots with 0% Zander decreased Na^+ concentration. However, with salinity stress, addition of extra water to pots with 0% and 10% Zander did not decrease Na^+ concentration of plants compared to pots without extra water. Water can ameliorate soil properties and improve uptake of nutrients. Moreover, addition of extra water to pots with 10% Zander without salt did not increase Ca^{++} concentration but addition of extra water to pots with 0% Zander did increase Ca^{++}. Saline irrigation applied as one application of 1.0 M NaCl increased Ca^{++} concentration in plants. With irrigation with extra water, Zander at 0% or 10% increased Ca^{++} concentration of plants compared with plants without salt. Overall addition of extra water is important in alleviating salt stress for soil under salt conditions. K^+ and Ca^{++} were significantly greater with increased level of Zander and addition of water. This agrees with the conclusion of Darwish, Persaud, and Martens (1995) who found uptake of major plant nutrients is mediated by organic matter. With application of water, Na^+ concentration decreased in plants but remained very high in the control. Saline stress, applied as one application of 1.0 M NaCl produced no significant change in Mg^{++} concentrations with 0% and 10% Zander but it was significantly lowered with 10% Zander. However, there was no significant change in Mg^{++} and P of plants compared to treatments without extra water. Saline stress, applied as one application of 1.0 M NaCl produced no significant change and less availability in Mg^{++} and P concentrations with 0% and 10% Zander. The additional irrigation water caused an increase in the ratio of Ca^{++}/Na^+ and K^+/Na^+ ratio with and without salt. Thus beneficial effect of Zander on salt tolerance can be partly reproduced by increased of supplying water to increase Ca^{++} but it remains unclear wheter this is due to increased water directly or indirectly to the effect extra water availability has on Ca^{++} uptake.

6.6 Conclusions

Application of Zander at 10 or 30% improved both physical and chemical properties of the soil, soil texture, and water holding capacity and nutritional value which increased plant establishment.

Extra water improved survival and number of leaves to the same level as 30% Zander and no extra water. Irrigation with 1.0 M NaCl and extra water increased survival and number of leaves, extra water also improved stem height, leaf area, shoot and root fresh and dry weight.

Addition of extra water at 0% Zander decreased Na^{+} concentration in plants, with salinity stress, while extra water to pots with 10% Zander did not decrease Na^{+} concentration. It is clear from the results that Ca^{++} is significantly affected by water with and without salt. The additional of irrigation water caused an increase in the uptake of Ca^{++}. Extra water increased K^{+} concentration with saline irrigation but not to the same level as 30% Zander. Furthermore, extra water with and without saline irrigation did not increase Mg^{++} and P concentration in plants, while extra water increased the Ca^{++}/Na^{+} and K^{+}/Na^{+} ratio.

Extra water was not completely efficient in correcting the detrimental effects of salt, but was able to mitigate them. The results with *A. saligna* demonstrated highly significant growth increased with Zander or irrigation water with have been due to an efficient utilization of soil nutrients when irrigation treatments removed the condition of moisture stress in the soil. This was evident from the significant interaction of applied Zander with amount of irrigation.

Chapter 7

Effects of Zander and additional calcium on mineral uptake and growth of *Acacia saligna* under saline conditions

7.1 Introduction

Improving soil conditions and establishing equilibrium among plant nutrients are important for soil productivity and plant production. Usually saline conditions are characterized by low nutrient ion activities and extreme ratios of Na^+/Ca^{++} and Na^+/K^+ resulting in nutritional disorders as well as reduced crop growth (Pessarakli 1994). Irrigated agriculture using saline water in arid and semi-arid regions may lead to salt build up in the soil and a reduction in yield and soil resource sustainability if proper management practices are not followed. The salinization of soils and water places substantial constraints on crop productivity in the arid and semi-arid regions (Royo *et al*. 2000). Applying an amendment to the salt affected soils can decrease the adverse effects of salinity and support plant growth. Zander is an organic deposit laid down over several thousand years (Zander Corporation 2007). Zander can affect growth, dry matter and yield. Martinez (2006) reported that dry weight of shoots is affected by the increase of salt concentration and dry mass of plants is increased in proportion to the increased level of Zander. As demonstrated by earlier results, in this study the decrease in growth of *Acacia saligna* grown in medium without Zander may be related to salt induced disturbance of the plant water balance, and in the extreme to loss of leaf turgor which can reduce leaf expansion and therefore, photosynthetic leaf area (Erdel and Taleisnik 1993, Huang and Redmann 1995). Other causes of growth reduction under salinity stress include ionic imbalances, change in nutrients, change in physiological processes and

biochemical reactions or a combination of such factors (Volkmar, Hu, and Steppuhn 1998, Hasegawa *et al*. 2000). Since Ca^{++} plays a vital nutritional and physiological role in plant growth owing to its effects on specific ions or Na^{+}/Ca^{++} balance, the effect of salinity may be ameliorated using calcium that is present in Zander. The addition of Zander stimulates significant plant growth under saline conditions as a result of the interaction between Na^{+} and Ca^{++} on plant growth (Rengel 1992). Many studies have indicated that the primary effect of salt stress is the disruption of membrane integrity caused by the displacement of Ca^{++} from the cell surface by Na^{+} (Cramer, Lauchli, and Epstein 1986, Lynch, Cramer, and Lauchli 1987) provided evidence for the displacement of membrane associated Ca^{++} by Na^{+} in root hairs of salinized cotton seedlings. A widespread practice to reduce the salt content in the soil is leaching. However, excess irrigation in order to leach salts is already becoming a less reasonable or acceptable option owing to the cost and lack of availability of water. One possible approach to reduce the effect of salinity on plant productivity is through the addition of Zander which is regarded as rich in calcium. Calcium is well known to have regulatory roles in plant metabolism (Cramer, Lauchli, and Epstein 1986) and Na^{+} ions may compete with calcium ions for membrane binding sites. It has therefore, been hypothesized that high calcium levels can protect the cell membrane from the adverse effects of salinity (Busch 1995). High calcium levels were found to protect the cells of the maritime halophyte *Aster tripolium* L. from the adverse effects of salinity (Perera, Robinson, and Mansfield 1995) and Lynch, Cramer, and Lauchli (1987) observed that 10 mM calcium was able to minimize the detrimental effects of salinity on root growth in cotton exposed to 75 mM NaCl. Externally supplied Ca^{++} has been shown to ameliorate adverse effects in plants presumably by facilitating higher K^{+}/Na^{+} selectivity (Hasegawa *et al*. 2000). However, calcium supplements were unable to ameliorate NaCl

damage in blueberry (*Vaccinium ashei* L.) and sunflower (*Helianthus annuus* L.) Sohan, Jasoni and Zajicek (1999), an alternative strategy for coping with salinity could be to supplement calcium where the growth medium is saline, although a wide range of investigations has been carried out on the effect of salinity in a number of crops.

The objective of this study was to test the hypothesis that supplementation of saline medium with calcium at the same level as is naturally found in 30% Zander can at least partially remediate the adverse effects of salinity on plant growth, leaf area, and mineral uptake in *A. saligna* and that part of the beneficial effect of Zander under saline condition is thus due to its high level of calcium.

7.2 Materials and methods

The experiment was conducted at the Experimental Station of the Faculty of Agriculture, Sidi El Mesri, Tripoli, Libya, from January to March 2009. Environmental data are given in Appendix 1. Seeds of *Acacia saligna* were obtained from Setropa BV, Troelstralaen 4, 1272 JZ Huizen, The Netherlands, and Zander was obtained from the Zander Corporation, UK. Zander was mixed with soil. Three different levels of Zander (0%, 10% and 30%) were applied in this study and only one concentration of salt irrigation and $CaCl_2$ mixed with soil and soil with Zander. After collecting the soil it was air dried and sieved through a 2 mm mesh sieve. Zander was added to soil at three different rates, 0%, 10% and 30% by weight. The nutrient solution for plants subjected to salinity stress was identical to that of the control except for the addition of NaCl. Seeds of *A. saligna* were sown in pots with a minimum diameter of 130 mm. The bottom of the pots contained a piece of filter paper to prevent the soil falling out when dry. Seeds were soaked by using 100 ml of boiling water poured onto 80 seeds for each

treatment and left to cool; boiling water was applied three times for 30 min and left to cool as described in Chapter 3. After that, the seeds were checked to see if they had imbibed. Thereafter, 20 imbibed seeds were sown in each pot. The soil material used in this study was taken from a surface horizon of Sidi El Mesri described in Chapter 7. Table 6.1 shows some of characteristics of the soil used. The design employed in this experiment was a Randomized Block Design (RBD) with four replicates of each treatment. Calcium content with 30% Zander was 0.67 g in 150 g Zander + 350 g dry soil, with 10% Zander Ca^{++} was 0.32 g with 50 g Zander + 450 g dry soil and with 0% Zander it was 0.11 g Ca^{++} with 500 g dry soil alone. The extra Ca^{++} required with 30% Zander mix was added to 0% or 10% Zander using $CaCl_2$.

Calculation of Ca^{++} from $CaCl_2$ used the equation

$$CaCl_2 = \frac{MW\ CaCl_2 \times Extra\ Ca\ (g)}{MW\ Ca^{++}}$$

Where 1 M $CaCl_2$ = 111 g l^{-1} MW of Ca^{++} = 40

Pots with 0% Zander had 1.55 g $CaCl_2$ and these with 10% Zander received 0.97 g $CaCl_2$ mixed in order to provide Ca^{++} equivalent to the Ca^{++} at 30% Zander. The treatment consisted of a control, plus four levels. The treatments used were 30% Zander, 10% Zander and 0% Zander without $CaCl_2$ and 10% Zander + 0.96 g of $CaCl_2$, 0% Zander + 1.53 g $CaCl_2$ in each pot, without salt irrigation. Another set of treatments were irrigated with 50 ml of 1.0 M NaCl once to 30% Zander, 10% Zander, and 0% Zander without $CaCl_2$ and with extra Ca^{++} (10% Zander) in each pot.

7.2.1 Plant growth measurement

At the end of the experiment seven weeks from the start of treatments, plant survival and the height of each individual plant was measured using a ruler. The plants were then divided into shoot and root components and the number of leaves were recorded. The roots were separated from the growth medium and washed in running water. Total leaf area per plant was measured with a Li-300 Leaf area meter (LI- COR. Inc., Lincoln, NE, USA). Fresh weights of shoots and roots were determined and dry weight was obtained after oven drying the sample at 80^{o}C.

7.2.2 Chemical analysis

At the end of the experiment plant samples were analyzed for Na, K, Mg, Ca and P. After rinsing with distilled water the samples were dried in an oven at 80^{o}C for 24 h and samples ground to pass through a 2 mm sieve; 0.5 g of each sample was than placed in a plastic bag. The dried plant materials from each plastic bag were digested with nitric acid and the amount of Na, K, Mg, Ca and P ions determined by ICP analysis using the same methods as described in Chapter 4.

7.2.3 Statistical analysis

The significance of differences between means was tested by three-way analysis of variance using Minitab Computer 15 Package. Significance was set at $p \leq 0.05$. Tukey's test was used for the mean comparison. Final % survival data was arcsin transformed before analysis.

7.3 Results

7.3.1 Plant Survival

Figure 7.1 show that in the absence of salinity, survival of plants was not significantly increased with 10% Zander compared with 0% Zander, but was significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander increased survival to the same level as with 30% Zander. Saline stress, applied as one application of 1.0 M NaCl significantly decreased plant survival with 0% and 10% Zander while survival was not significantly affected with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander increased survival to the same level as with 30% Zander and no extra Ca^{++}. With Ca^{++} applied there was no significant difference between treatments regardless of level of Zander.

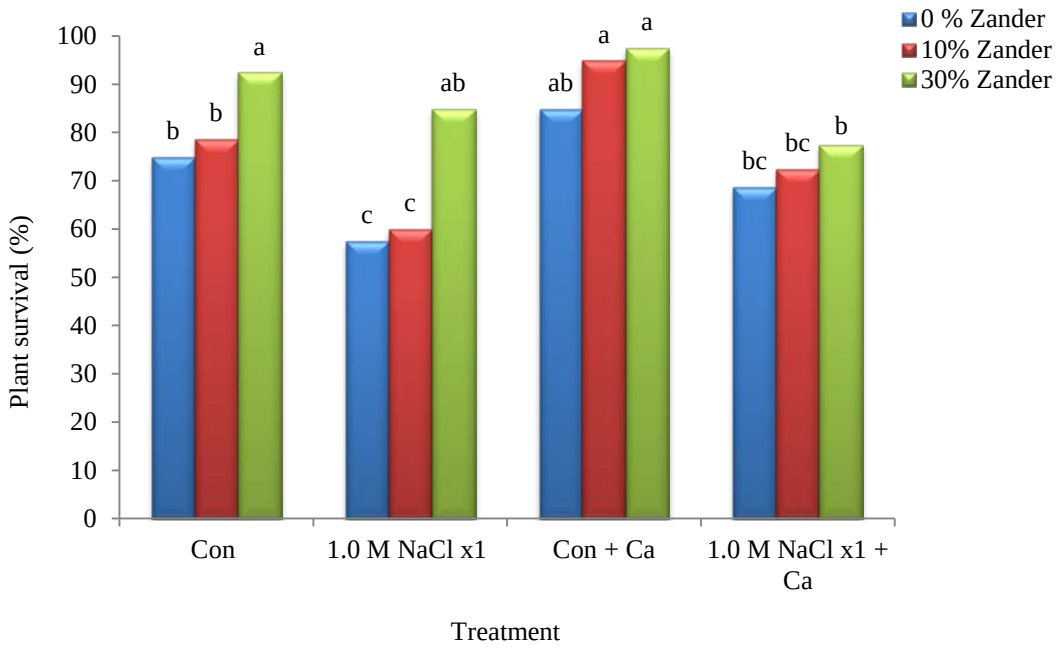


Figure 7.1: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on the survival of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

7.3.2 Shoot height, leaf number and leaf area

Figure 7.2 shows that in the absence of salinity stress, Zander at 10% significantly increased shoot height compared with 0% Zander and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander significantly increased shoot height. However, height was still significantly below that with 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased shoot height with all levels of Zander. With saline stress, Zander at 10% significantly increased shoot height compared with treatments without Zander, and this was further significantly increased with 30% Zander. With saline stress, addition of extra Ca^{++} to pots with 10% Zander significantly increased shoot height to the same as with 30% Zander and no additional Ca^{++}. Addition of extra Ca^{++} to pots with 0% Zander also significantly increased shoot height, but not to the same level as 30% Zander and no extra Ca^{++}.

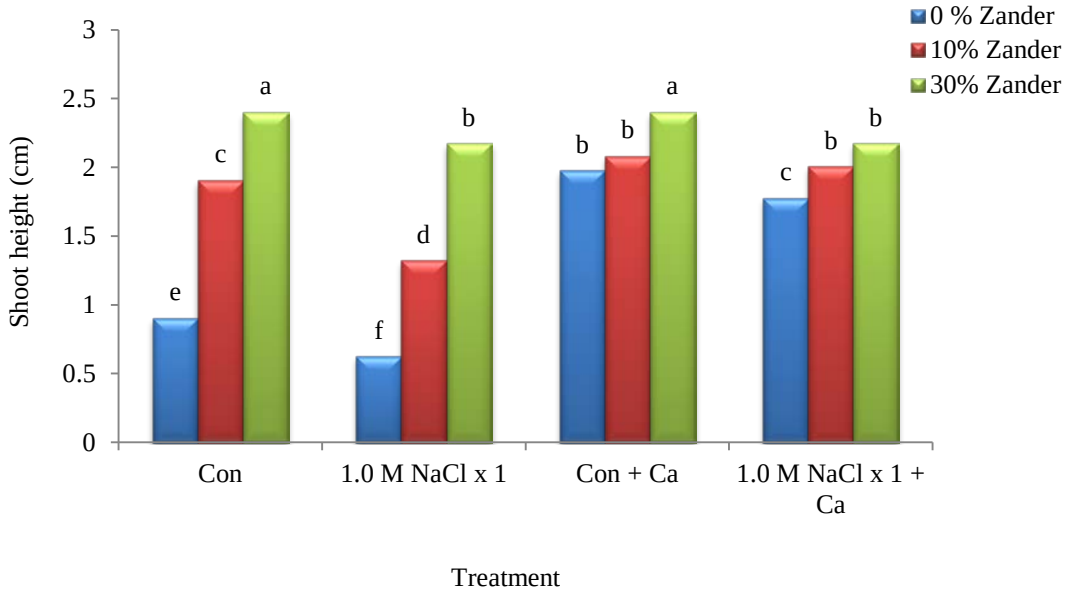


Figure 7.2: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot height of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 7.3 shows that in the absence of salinity stress, Zander at 10% significantly increased number of leaves and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% or 10% Zander did not significantly affect leaf number compared to those grown without Ca^{++}. Saline stress applied as one application of 1.0 M NaCl significantly decreased number of leaves of plants grown in soil alone (0% Zander) but number of leaves of plants grown with 10% or 30% Zander was not significantly affected. With saline stress, Zander at 10% significantly increased number of leaves compared with controls with no Zander and this was further significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots did not significantly increase leaf number compared to those grown without calcium or control plants without salinity or Ca^{++}. Moreover, leaf numbers in 0% and 10% Zander treatments remained lower than with 30% Zander and no extra Ca^{++}.

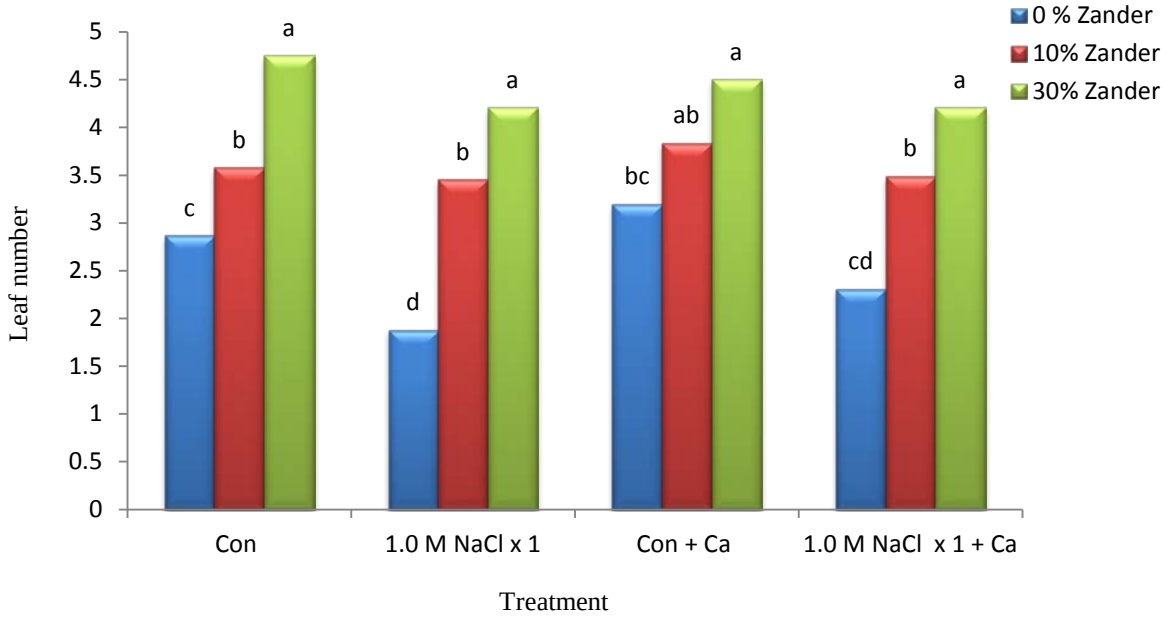


Figure 7.3: Effect of NaCl irrigation of 50 ml with1.0 M NaCl on leaf number of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 7.4 shows that in the absence of saline stress, addition of Zander at 10% to the soil significantly increased leaf area compared with 0% Zander, and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander increased leaf area to the same level as with 30% Zander and no extra Ca^{++}. Addition of extra Ca^{++} to pots with 0% Zander significantly increased leaf area but did not increase leaf area to the same level as with 30% Zander and no extra Ca^{++}. Saline stress applied as one application of 1.0 M NaCl significantly decreased leaf area for all levels of Zander. With saline stress Zander at 10% significantly increased leaf area and this was further significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander significantly increased leaf area such that there was no difference from treatments with saline stress 30% and additional Ca^{++}, Moreover, the addition of extra Ca^{++} to saline stressed plants resulted in any significant difference between treatments regardless of level of Zander.

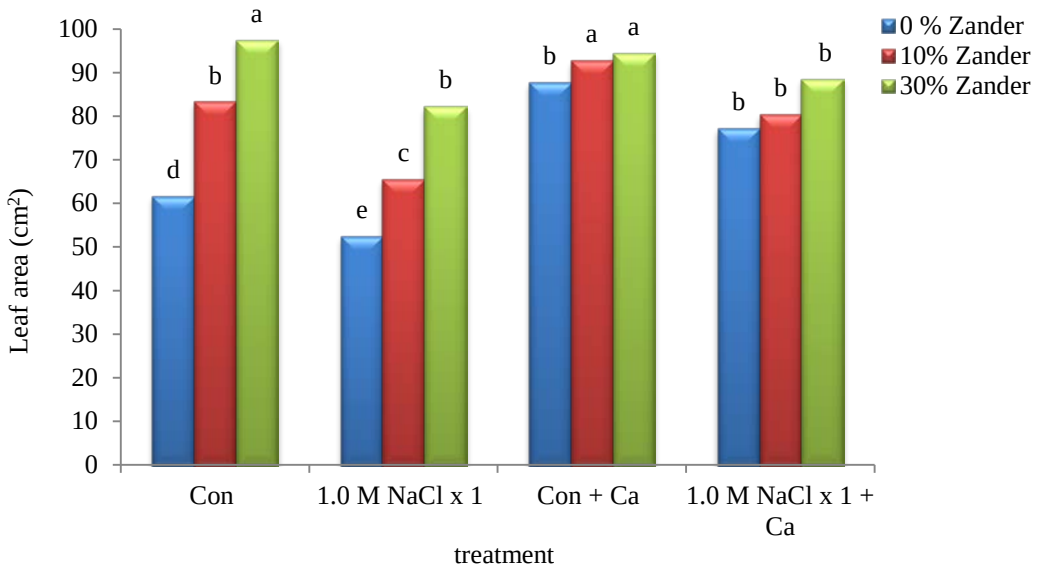


Figure 7.4: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on leaf area of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

7.3.3 Shoot fresh and dry weight

Figure 7.5 shows that in the absence of salinity stress, Zander at 10% significantly increased shoot fresh weight and this was further significantly increased with 30% Zander. Addition of extra Ca^{++} to pots with 0% Zander increased shoot fresh weight but not to the same level as with 30% Zander and no extra Ca^{++}. Shoot fresh weight grown with 10% Zander was unaffected by addition extra $Ca^{++,}$ but again was significantly lower than treatments with 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased shoot fresh weight for all levels of Zander. With saline stress, Zander at 10% significant increased shoot fresh weight compared to plants without Zander and this was further significantly increased with 30% Zander. With saline stress, addition of extra Ca^{++} to pots with 0% or 10% Zander did not affect shoot fresh weight compared with treatments without extra Ca^{++}, and the weight remained significantly below those with 30% Zander and no extra Ca^{++}.

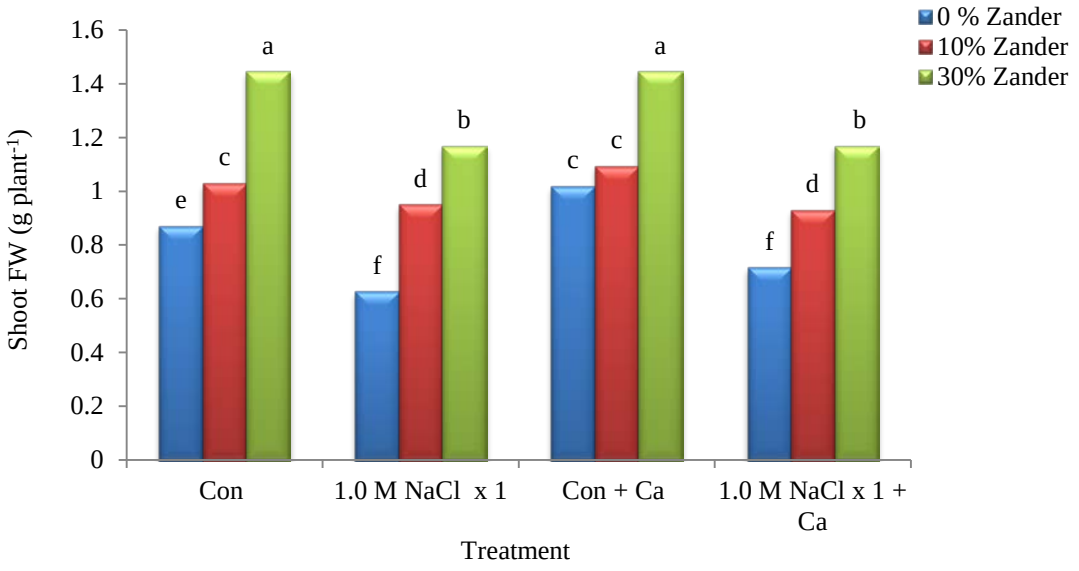


Figure 7.5: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot fresh weight of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 7.6 shows that in the absence of salinity stress, Zander at 10% significantly increased shoot dry weight and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander did not significantly increase shoot dry weight and this remained significantly below that of 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased shoot dry weight with all levels of Zander. With saline stress, Zander at 10% significantly increased shoot dry weight and was further increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander did not significantly alter shoot dry weight and this was significantly below that with 30% Zander and no extra Ca^{++}. Thus the addition of extra Ca^{++} appears to have had no significant affect on shoot dry weight.

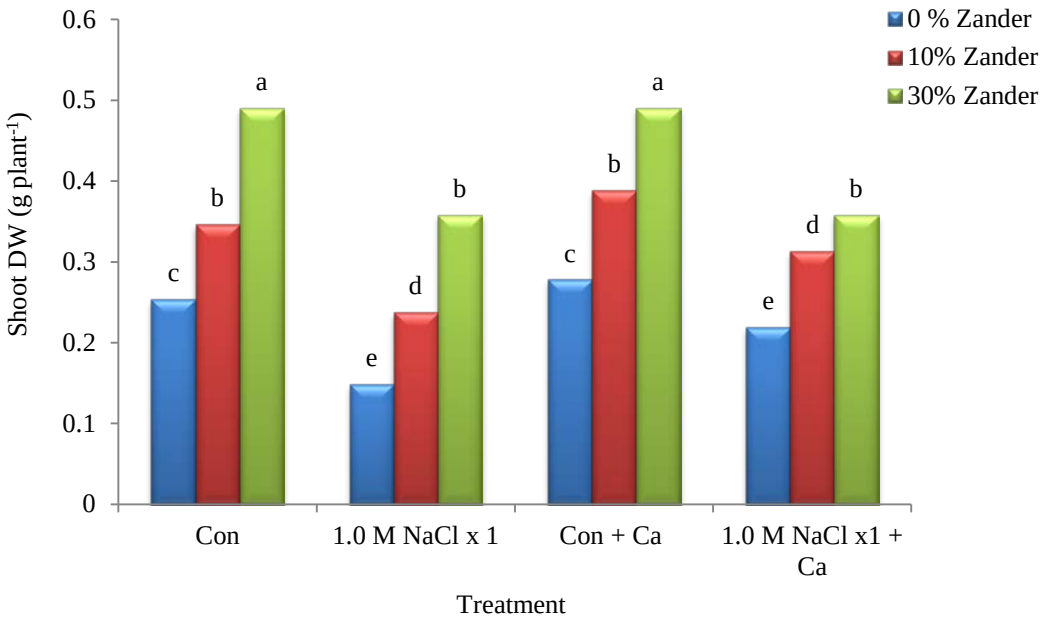


Figure 7.6: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot dry weight of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

7.3.4 Shoot moisture content

Figure 7.7 shows that in the absence of salinity stress, Zander at 10% significantly decreased shoot moisture content but this was unaffected with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander did not significantly change shoot moisture content and this remained significantly below the level with 30% Zander and no extra Ca^{++}. However, addition of extra Ca^{++} to pots with 0% Zander significantly increased shoot moisture content compared with treatments with 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly increased shoot moisture content with 0% and 10% Zander but not with 30% Zander. With saline stress, Zander at 10% and 30% showed significantly decreased shoot moisture content compared with no Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander had no significant affect on shoot moisture content.

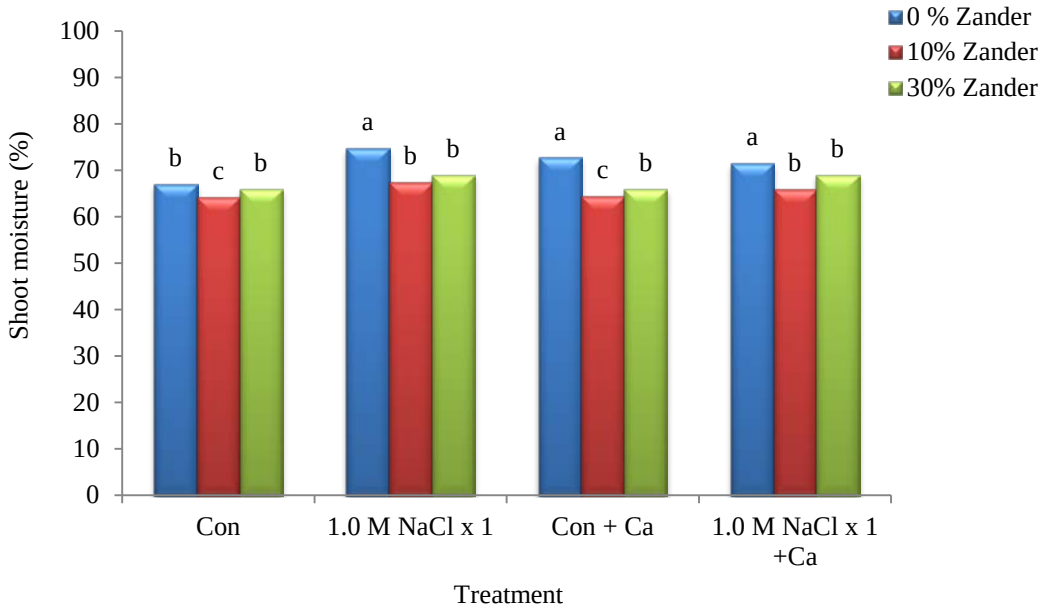


Figure 7.7: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on shoot moisture content of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

7.3.5 Root fresh and dry weight

Figure 7.8 shows that in the absence of salinity stress, root fresh weight did not significantly with 10% Zander and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander did not alter root fresh weight significantly and it remained significantly below the level with 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased root fresh weight for all levels of Zander. With saline stress, Zander at 10% significantly increased root fresh weight and this was further significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander did not significantly increase root fresh weight nor did it reach the same level as with 30% Zander and no extra Ca^{++}.

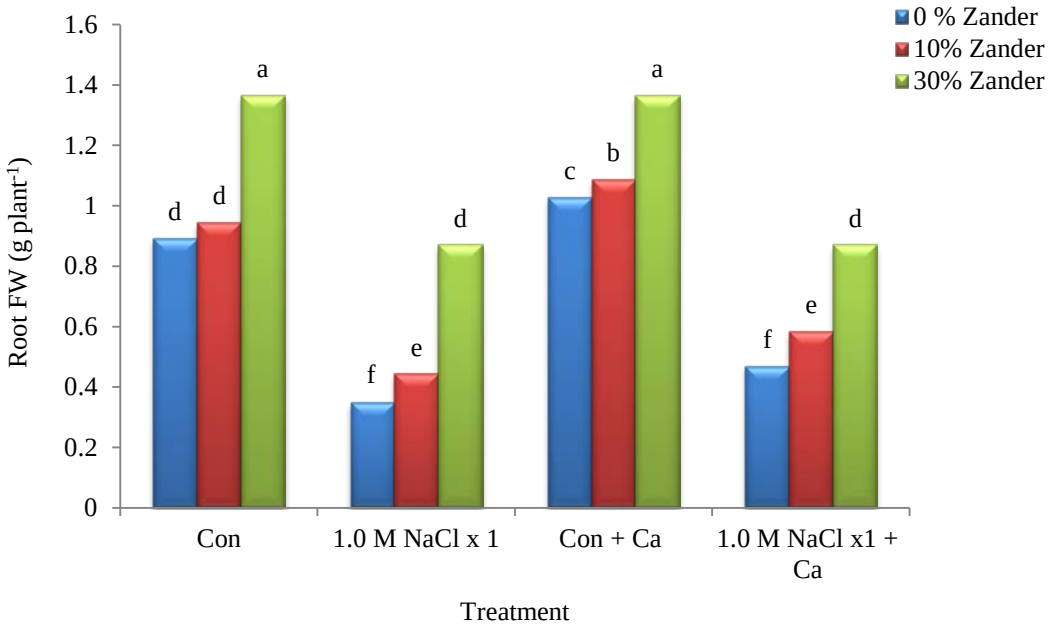


Figure 7.8: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on root fresh weight of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Figure 7.9 shows that in the absence of salinity stress, Zander at 10% significantly increased root dry weight and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander did not increased root dry weight to the same level as 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased root fresh weight with 10% and 30% Zander but there was no significantly affect with 0% Zander. With saline stress, Zander at 10% did not significantly increase root fresh weight compared to no Zander but this was significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 10% Zander increased root dry weight such that there was no significant difference from treatments with 30% Zander and no extra Ca^{++}. However, addition of extra Ca^{++} to pots with 0% Zander did not increase root dry weight to the same level as with 30% Zander and no extra Ca^{++}.

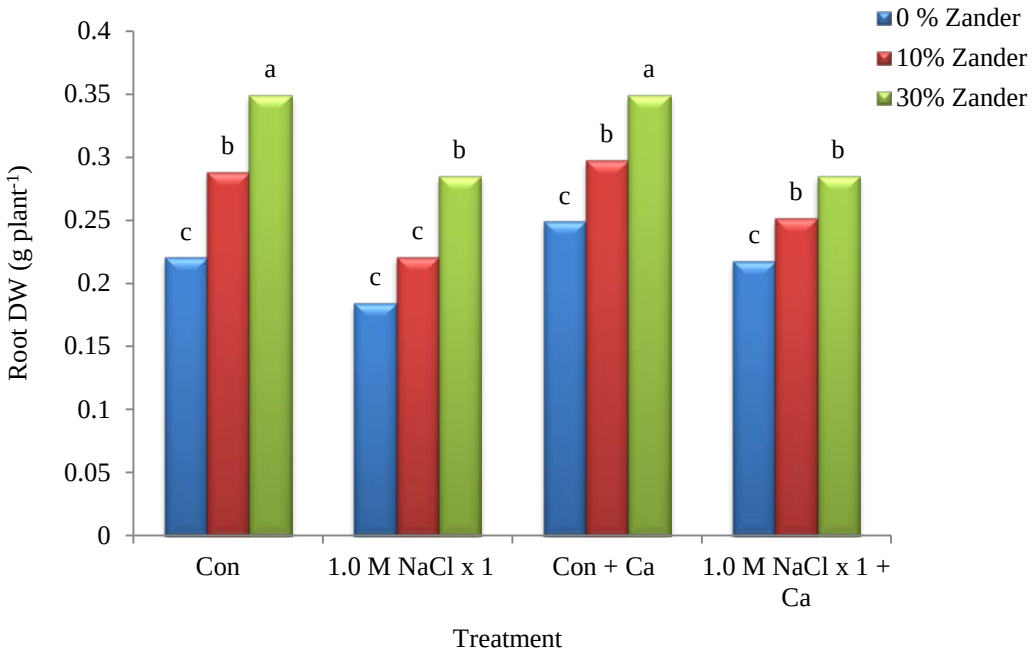


Figure 7.9: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on root dry weight of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya. Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

7.3.6 Root moisture content

Figure 7.10 shows that in the absence of salinity stress, Zander at 10% significantly increased root moisture content and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander significantly increase root moisture content to the same level as 30% Zander and no extra Ca^{++}. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% did not significantly increase root moisture content and it remained significantly below the level with 30% Zander and no extra Ca^{++} Saline stress, applied as one application of 1.0 M NaCl significantly decreased root moisture content with 0% and 10% Zander but not with 30% Zander. With saline stress, Zander and 10% significantly increased root moisture compared with no Zander and this was further significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander failed to significantly increase root moisture content and this remained significantly below the level with 30% Zander and no extra Ca^{++}.

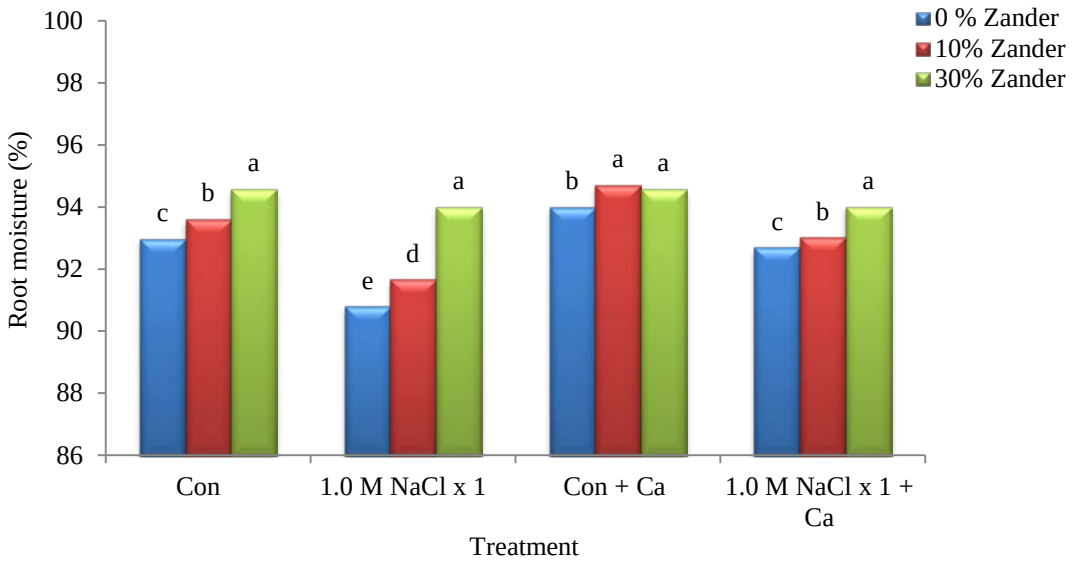


Figure 7.10**:** Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on root moisture content of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya**.** Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 7.1 shows the summary of beneficial effect of extra calcium on percentage survival and plant growth parameters of *Acacia saligna* grown in medium with extra calcium applied to pots in the field in Libya.

Table 7.1: Does extra calcium applied account for the beneficial effect of Zander on plant growth?

	No salt		With salt	
	0% Zander	10% Zander	0 % Zander	10% Zander
Survival %	+ +	+ +	+ +	+ +
Stem height	+	+	+	+ +
Leaf number	-	+ +	-	-
Leaf area	+	+ +	+ +	+ +
Shoot FW	+	-	-	-
Shoot DW	-	-	-	-
Shoot moisture	+	-	-	-
Root FW	-	-	-	-
Root DW	-	-	-	+ +
Root moisture	-	+ +	-	-

+ = Significant increase by addition of Ca^{++}

+ + = Significant increase to at least the same level as with 30% Zander and no extra calcium

Additional of extra Ca^{++} to 0% Zander play increased survival and leaf area of plants under saline condition to the same level with 30% Zander and no extra calcium. Part but not all of the effect of Zander may be explained by improved calcium supply

7.3.7 Effects of Zander and extra calcium on mineral content of plants

Table 7.2 shows that in the absence of salinity stress Na^+ in plants grown in 10% Zander was not significantly different from the Na^+ concentration of plants grown with 0% Zander, but this significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander did not significantly affect Na^+ concentration. Addition of extra Ca^{++} to pots with 0% Zander significantly reduced Na^+ concentration in plants. Saline stress, applied as one application of 1.0 M NaCl significantly increased Na^+ concentration in plants with all levels of Zander. With saline stress, Na^+ concentration with 10% Zander did not differ significantly compared with 0% Zander but Na^+ concentration was significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander significantly decreased concentration of Na^+ compared with no extra Ca^{++}. However, in both cases Na^+ concentration remained significantly below that of stressed plants in 30% Zander with or without extra Ca^{++}.

Table 7.2: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Na^+ content of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya

	Na^+ Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	241.1 ^{e}	298.9 ^{e}	405.8 ^{d}
1.0 M NaCl x 1	8992.3 ^{b}	10618.1 ^{b}	11529.9 ^{a}
Con + Ca^{++}	193.8 ^{f}	211.6 ^{e}	408.2 ^{d}
1.0 M x 1 NaCl + Ca^{++}	4688.6 ^{c}	4807.9 ^{c}	11515.4 ^{a}

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 7.3 shows that in the absence of salinity stress, Ca^{++} concentration in plants with 30% Zander was significantly greater than with 0% Zander and 10% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander significantly increased Ca^{++} concentration in plants to the same level as those with 30% Zander and no extra Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased Ca^{++} concentration will all levels of Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander increased Ca^{++} concentration in plants to the same level as with 30% Zander and no extra Ca^{++}. Thus, the addition of extra Ca^{++} replicated the beneficial effect of Zander on Ca^{++} concentration in plants.

Table 7.3: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Ca^{++} content of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya

	Ca^{++} Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	10840.9 [c]	14853.3 [b]	18891.0 [a]
1.0 M NaCl x 1	8662.0 [d]	12936.2 [c]	16109.4 [b]
Con + Ca^{++}	15230.9 [b]	17885.7 [a]	18784.5 [a]
1.0 M x 1 NaCl + Ca^{++}	13242.9 [b]	15434.8 [b]	16232.6 [b]

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 7.4 shows that addition of Zander at 10% to the soil, did not significantly affect K^+ concentration compared with plants grown with 0% Zander and these was no significant difference between 10% and 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander did not significantly increase K^+ concentration in plants and there was no significant difference from the level with 30% Zander and no extra Ca^{++}. Addition of extra Ca^{++} to pots with 0% Zander did not increase K^+ concentration in plants and this remained significantly below the level with

30% Zander and no extra Ca^{++}. Saline stress applied as one application of 1.0 M NaCl significantly decreased K^+ concentration with 0% Zander but did not significantly alter concentrations with 10% Zander. With salinity stress and addition of extra Ca^{++} to pots with 10% Zander, K^+ concentration in plants was not significantly different from that with 10% or 30% Zander and no extra Ca^{++}. Addition of extra Ca^{++} to soil alone did not significantly increase K^+, and it remained significantly below the level with 30% Zander and no extra Ca^{++}.

Table 7.4**:** Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on K^+ content of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya**.**

	K^+ Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	10199.3 [b]	12767.5 [ab]	15308.4 [a]
1.0 M NaCl x 1	6457.8 [c]	8828.5 [b]	11373.0 [b]
Con + Ca^{++}	11208.1 [b]	14462.3 [a]	15552.0 [a]
1.0 M x 1 NaCl + Ca^{++}	8905.4 [c]	10971.4 [b]	11257.7 [ab]

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 7.5 shows that the Mg^{++} concentration in plants in the absence of salinity stress, with Zander at 10% was significantly higher than with 0% Zander and this was further significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% Zander significantly increased Mg^{++} concentration but not with 10% Zander. Furthermore Mg^{++} remained significantly below that of plants grown in 30% Zander with no extra Ca^{++}. Saline stress applied as one application of 1.0 M NaCl significantly decreased Mg^{++} concentration with 0% and 10% Zander but not with 30% Zander. With saline stress, Zander at 10% significantly increased Mg^{++} compared with plants with no Zander, and this was further significantly increased with 30%

Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander significantly increased Mg^{++} concentration but this remained significantly below the concentration with 30% Zander and no extra Ca^{++}. Thus, the addition of extra Ca^{++} partly but not completely replicated the beneficial effect of Zander on Mg^{++} concentration in plants.

Table 7.5: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Mg^{++} content of *Acacia saligna* growing in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya

	Mg^{++} Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	2522.7 [c]	2968.6 [b]	3375.2 [a]
1.0 M NaCl x 1	1906.6 [d]	2281.8 [c]	3025.9 [a]
Con + Ca^{++}	2889.1 [b]	3003.4 [b]	3337.6 [a]
1.0 M x 1 NaCl + Ca^{++}	2515.7 [c]	2884.8 [b]	3122.5 [a]

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 7.6 shows that in the absence of salinity stress, addition of 10% Zander to the soil did not significantly increased P concentration in plants but this was significantly increased with 30% Zander. In the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander significantly increased P to the same level as with 30% Zander and no extra Ca^{++}. However, addition of extra Ca^{++} to pots with 0% Zander did not significantly increase P concentration of plants to the same level as 30% Zander and no Ca^{++}. Saline stress, applied as one application of 1.0 M NaCl significantly decreased P concentration with all levels of Zander. With saline stress, Zander at 10% significantly increased P of plants compared to plants with no Zander and this was further significantly increased with 30% Zander. With salinity stress, addition of extra Ca^{++} to

pots with 0% and 10% Zander significantly increased P concentration in plants but not to the same level as with 30% Zander and no extra Ca^{++}.

Table 7.6: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on P content of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya.

	P Concentration (mg kg^{-1})		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	1339.2 ^{bc}	1651.2 ^{b}	2338.4 ^{a}
1.0 M NaCl x 1	444.9 ^{e}	815.3 ^{d}	1585.8 ^{b}
Con + Ca^{++}	1503.8 ^{b}	2128.7 ^{ab}	2396.1 ^{a}
1.0 M x 1 NaCl + Ca^{++}	859.6 ^{d}	1301.5 ^{c}	1538.1 ^{b}

Means with the same letter are not significantly different at $p \leq 0.05$ based on three-way analysis of variance and using Tukey's test.

Table 7.7 and 7.8 shows that in control of plants with 10% and 30% Zander, Zander increased K^{+}/Na^{+} and Ca^{++}/Na^{+} ratios. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander increased K^{+}/Na^{+} and Ca^{++}/Na^{+} ratios and these were higher than that with 30% Zander and no Ca^{++}. Saline stress decreased K^{+}/Na^{+} and Ca^{++}/Na^{+} with all levels of Zander. With salinity stress, addition of extra Ca^{++} to pots with 0% and 10% Zander increased K^{+}/Na^{+} and Ca^{++}/Na^{+} ratios and this was higher than that with 30% Zander and no extra Ca^{++}.

Table 7.7: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on K^+ /Na^+ ratio of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya.

	K^+ /Na^+		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	42.3	42.70	37.72
1.0 M NaCl x 1	0.72	0.83	0.99
Con + Ca^{++}	57.83	68.35	38.09
1.0 M x 1 NaCl + Ca^{++}	1.91	2.28	0.98

Table 7.8: Effect of NaCl irrigation of 50 ml with 1.0 M NaCl on Ca^{++} /Na^+ ratio of *Acacia saligna* grown in medium with three different concentrations of Zander and extra calcium supplement in the field in Libya.

	Ca^{++}/Na^+		
Treatment	0% Zander	10% Zander	30% Zander
Con (No salt)	44.97	49.68	46.55
1.0 M NaCl x 1	0.96	1.22	1.40
Con + Ca^{++}	78.58	84.53	46.01
1.0 M x 1 NaCl + Ca^{++}	2.82	3.21	1.41

Zander increases uptake of Ca^+ and K^+ by *A. saligna* and also increased Ca^{++}/Na^+ and K^+/ Na^+ ratios. The results indicate that the beneficial effect of Zander on salt tolerance can be partly reproduced by supplying Ca^{++} in the absence of the organic material itself. The hypothesis calcium improves salinity tolerance by increasing uptake of protective Ca^{++} and K^+ ions and additional of calcium increased K^+/Na^+ and Ca^{++}/ Na^+ ratio.

Table 7.9 shows the summary of beneficial effect of calcium on nutrition content of *Acacia saligna* grown in medium with extra calcium applied to pots in the field in Libya.

Table 7.9: Does extra calcium applied account for the beneficial effect of Zander on the ion uptake?

	No salt		With salt	
	0% Zander	10% Zander	0 % Zander	10% Zander
Na+	+	+	+	+
Ca^{++}	+	++	+ +	+ +
K^{+}	-	-	-	+
Mg^{++}	+	-	+	+
P	-	-	+	+
Ca^{++}/ Na^{+}	+ +	+ +	+ +	+ +
K^{+}/Na^{+}	+ +	+ +	+ +	+ +

+ = Significant increase by addition of Ca^{++}

+ + = Significant increase to at least to the same level as with 30% Zander and no extra calcium.

Additional of extra Ca^{++} to 0% Zander plays a significant role in Ca^{++} uptake and Ca^{++}/Na^{+} and K^{+}/Na^{+} ratios increasing them the same level as with 30% Zander and no extra calcium. Part but not all of the effect of Zander may be explained by improved calcium supply.

7.4 Discussion

Calcium is a non-toxic inorganic nutrient that is very effective in detoxifying high concentrations of other elements in plants under saline conditions (Greenway and Munns 1980). The hypothesis tested in this research is that Zander improves salinity tolerance by increasing uptake of Ca^{++} and results suggest that the beneficial effects of Zander on survival and plant growth can be reproduced by increasing the Ca^{++} concentration in plants to the level found in Zander by the application of $CaCl_2$.

Salt stress had a significant inhibitory effect on plant survival and fresh and dry weight of shoots and roots. Plants grown in 30% Zander differed significantly in shoot dry weight was obtained in their response with less reduction observed in the absence of salinity.

Fresh and dry weight of shoots decreased with irrigation with saline water and these results agrees with Martinez (2006) who reported that the fresh and dry weight of shoots is affected by the amount of salt water irrigation. The fresh and dry weight of shoots with and without salt was not affected by the extra Ca^{++}. The variations in fresh and dry weight at 0 and 1.0 M NaCl were less evident in the media with 0% and 10% Zander. Addition of extra Ca^{++} to pots with 0% and 10% Zander did not increase root fresh and dry weight. Similarly supplementary Ca^{++} was found to enhance dry matter production in tomato (Navarro *et al*. 2000), strawberry (Kaya *et al*. 2002).

A reduction in moisture content was observed in salt stressed plants (Figure 7.7). The decrease in water moisture content indicated a loss of turgor that resulted in limited water availability for cell extension processes. Thus the growth inhibition in *A. saligna*

could be related to the decrease of moisture content induced by salt treatment. Several studies have shown that water uptake, and hence water content in the plants declined as the salt concentration in the irrigation water increased (Soria and Cuartero 1997, Bayuelo-Jimenez, Debouck, and Lynch, 2003, Cabanero, Martinez, and Carvajal, 2004).

Extra Ca^{++} with saline stress and Zander at 10% increased root moisture to the same level to the same level as with 30% Zander while with and without salinity stress, Ca^{++} applied to pots with 0% Zander did not increase in root moisture content. These results are in support of the initial hypothesis that extra supply of calcium ameliorated the effect of salinity, as has been found in cucumber (Kaya, *at al*. 2002) and pepper (Cabanero, Martinez, and Carvajal, 2004). These studies reported that NaCl decreased the passage of water through the membrane and roots reducing the activity of aquaporins, and that Ca^{++} ameliorated the negative effect of NaCl stress. Aquaporins allow water to pass freely across cellular membranes, following osmotic or hydrostatic pressure gradients (Chispeels and Maurel, 1994).

The results indicate that the beneficial effect of Zander on salt tolerance can be partly reproduced by supplying Ca^{++} in the absence of the organic material itself at least for survival and leaf area. However, other beneficial effects of Zander on plant growth cannot fully be reproduced by increasing the Ca^{++} concentration in the plants to the level found with Zander, by application of $CaCl_2$.

High concentration of sodium has a negative impact on other ions such as K^{+}, Mg^{++}, Ca^{++}, and P which are very important for the growth of plants. The possible cause of reduced nutrient uptake under salinity is that ions present in high concentration in the

external solution (i.e. Na^+ or Cl^-) are taken up at a high rate, which may lead to excessive accumulation in the tissue, these ions may inhibit the uptake of other ions into the roots and their transport into the shoot eventually leading to deficiency in the tissue. Rengel (1992) reported that the primary response of plants to salt stress is a change in the Ca^{++} homeostasis. He attributed the salt tolerance of plants to their ability to avoid Na^+ toxicity and to maintain Ca^{++} and K^+ levels. Salt-induced nutrient deficiency has been reported by many researchers (Sultana, Ikeda, and Kashem, 2001, Kaya *et al*. 2002). Na^+ is not considering an essential element for plants and plants accumulate Na^+ at the expense of Ca^{++} and K^+ in saline conditions (Kuiper, 1984). Na^+ may help in maintain the turgor but it is unable to substitute for the specific functions of Ca^{++} and K^+, for example enzyme activation and protein synthesis to give adequate growth (Leigh and Storey, 1991). The present results show that in the absence of salinity stress, addition of extra Ca^{++} to pots with 10% Zander increased Ca^{++} concentration in plants to the same level as those with 30% Zander and no extra Ca^{++}. Extra Ca^{++} with saline stress and Zander at 0% and 10% increased Ca^{++} concentration in plants to the same level as those with 30% Zander and no extra Ca^{++}.

There was no significant difference in K^+ concentration between plants grown with extra Ca^{++} with and without saline stress and those grown in Zander. As the Ca^{++} concentration of the plants increased K^+ concentration decreased. Ben-Hayyim, Kafkafi, and Gamore-Neumann (1987) found similar results in cultured *Citrud sinesis* cells in which the internal K^+ concentration decreased with an increased level of Ca^{++}, and concluded that K^+ probably plays a key role in the determination of growth.

Phosphorus is an important nutrient and its concentration in plant tissue is recognised to decline with increase of NaCl (Sharpley, Meisinger, and Suarez, 1992). There was no significant change in P concentration in plants without salt with 0% or 10% Zander and extra Ca^{++}. However, with plants grown in 0% or 10% Zander the concentration of P increased but not to the same level as with 30% Zander and no extra Ca^{++}.

Magnesium is an extremely important element for plants, notably in photosynthesis. Mg^{++} is involved in enzyme reactions. Mg^{++} concentration in plants in the absence of salinity stress, with Zander at 0% was higher than with 10% Zander with extra Ca^{++} applied. In the absence of salinity stress, addition of extra Ca^{++} to pots with 0% or 10% Zander significantly increased Mg^{++} concentration but not to the same level as with 30% Zander and no extra calcium.

The results for Ca^{++}/Na^{+} and K^{+}/Na^{+} ratios suggest that Ca^{++} may have played an important role in maintaining the proper function of biological membranes and their permeability (Kent and Lauchli, 1985), thereby resulting in relatively normal plant growth. The ability to toxic Na^{+} from the leaf lamina combined with the ability to maintain relatively high Ca^{++} concentration in leaves may provide *A. saligna* with a mechanism for maintaining low and moderate salinity levels. Ebert, *et al*. (2002) pointed out that cation relations in the shoot tissue such as Ca^{++}/Na^{+} have a stronger influence on salt tolerance than absolute sodium levels. Presence of higher level of Ca^{++} induced signals and the concentration difference across the plasma membrane results in a very steep electrochemical gradient in favour of Ca^{++} influx. Concentration of ions which are significantly higher or lower than this optimum concentration may decrease metabolic and hormonal function (Flowers and Lauchli, 1983).

The results indicate that the beneficial effect of Zander on salt tolerance can be partly reproduced by supplying Ca^{++} in the absence of the organic material itself. NaCl concentration in the nutrient solution reduced plant growth, and application of extra Ca^{++} gave better overall results than 0% and 10% Zander. With extra Ca^{++} there was an in increased fresh and dry weight of *A. saligna* grown under saline condition, and in terms of plant survival, number of leaves, shoot height, leaf area, as well as moisture content.

Extra Ca^{++} added to pots with 0% and 10% Zander decreased concentration of Na^{+} and increased Ca^{++} concentration in plants to the same level as with 30% Zander. These results confirm those of Ben-Hayyim, Kafkafi, and Gamore-Neumann (1987) who found that Na^{+} uptake was reduced with increased Ca^{++} concentration and this ability to avoid the toxicity of Na^{+} by increased growth of plants and this appears to be a further example of antagonism. Extra Ca^{++} did not increase K^{+} and Mg^{++} concentration in plants but did increase concentration of P. It is possible that Ca^{++} affects membrane permeability and inhibits Na^{+} uptake (Zhong and Lauchli, 1993).

The experiments clearly demonstrated the beneficial effects of Ca^{++} application on perennial *A. saligna* growth under saline stress. It should be possible to ameliorate the deleterious effect of salinity by supplying further Ca^{++} to either the irrigation water or saline soil. It also appeared that foliar application of Ca^{++} in saline stress with 0% Zander is more effective than 10% Zander. Moreover, further increase of Ca^{++} concentration at the increased salinity concentration is improved the growth and nutritional status of *A. saligna*.

These results obtained suggest and confirm, that the additional Ca^{++} may reduce uptake of Na^{+} by the plants.

7.5 Conclusions

NaCl concentration in the nutrient solution reduced plant growth. Application of Zander at 30% gave better overall results than 0% and 10% Zander. Extra Ca^{++} had no effect on shoots and roots fresh and dry weight of *A. saligna* grown under saline conditions.

The results for this study clearly show that addition of extra Ca^{++} to 0% Zander produced the same effect on survival, leaf area and stem height as with as 30% Zander and no extra calcium. Part but not all of the effect of Zander can be explained by improved calcium supply.

Addition of extra Ca^{++} at 0% Zander decreased Na^{+} concentration in plants, with salinity stress, while extra Ca^{++} to pots with 10% Zander did not decrease Na^{+} concentration. Addition of extra Ca^{++} to pots with 0% and 10% Zander increased Ca^{++} concentration in plants to the same level as with 30% Zander but did not increase K^{+}, and Mg^{++} concentration, With extra Ca^{++} phosphorus become more available in the soil. Extra Ca^{++} applied increased the Ca^{++}/Na^{+} and K^{+}/Na^{+} ratios. The beneficial effect of Zander on salt tolerance can be partly reproduced by supplying Ca^{++} in the absence of the organic material itself.

Results suggest that the beneficial effects of Zander on survival and plant growth can be reproduced by increasing the Ca^{++} concentration in the plants to the level found with Zander by the application of $CaCl_2$ through the irrigation water but that the beneficial effect of Zander can be partially reproduced by additional irrigation, supporting the second hypothesis above.

Chapter 8

General discussion, conclusions, recommendations and future work

8.1 Discussion

Many parts of the newly reclaimed areas in the north of Libya now face the problem of increasing salinity. The crop area affected by salinity is about 1.5 million hectares, in addition to 2 million ha used as pasture. The soil is facing the problem of salinity especially where the ground water contains 1500 ppm or more of soluble salts. The presence of soluble salts in the soil may affect plant growth in different ways. The high concentrations of specific ions can induce physiological disorders due to toxicity.

Acacia saligna produce high quality fodder which grows with low rainfall in dry regions. Plants can be utilized all year and can bridge the forage scarcity of annual dry seasons. *Acacia saligna* is a legume which grows usually as a tree or shrub. The relatively deep roots of these woody perennials allow them to reach soil nutrients and moisture not available to grasses and herbaceous plants. This characteristic enables these plants to retain fresh foliage into the dry season in Libya. Another reason for its choice as a fodder species is the ability of *Acacia saligna* to fix atmospheric nitrogen which makes it a protein rich feeds.

This study aimed to investigate some major factors affecting establishment under irrigation with NaCl, pre-treatments of seed to overcome seed coat dormancy and depth of sowing. The study aimed also to elucidate, if possible, the role of the organic

material called "Zander" in increasing water holding capacity and improving nutrient provision leading to improved establishment of *A. saligna* under saline conditions.

8.1.1 Germination

The hard seed dormancy of *Acacia* species impedes initiation of the germination process (Khasa 1993). To accelerate its germination, *Acacia* seeds should be pre-treated with one of several methods, amongst which are soaking in boiling water scarification with sandpaper and soaking in concentrated acids to break down or soften the seed coat. Both boiling water treatment and scarification had a clear positive impact on germination; pre-treatment increased germination of *A. saligna* up to 90%. Immersion of seeds in boiling water may stimulate germination by causing rupture of the seed coat, thereby allowing water to enter the seeds as reported by Willian (1985) and Holmes, McDonald, and Juritz (1987). The results indicated that treatment of *A. saligna* with boiling water more than three times is not worthwhile. This finding agrees with that reported by Omori (1993). Seeds made water permeable by the boiling water treatment germinated at a much slower rate than those made water permeable by mechanical scarification. However, the boiling treatments make it easier and safe to treat a large number of seeds in this way although treatment by scarification was also successful and gave 90% germination of seeds.

8.1.2 Seed size and sowing depth

Seed size may be an important factor affecting seedling survival as it is likely affect the quantity of metabolic reserves in the seed and size of embryos which, in turn, affects

vigour and establishment of the resultant seedling (Bonfil 1998). Large seeds germinated earlier and achieved greater germination than smaller and medium seeds. Sowing *A. saligna* seeds at 30 mm depth gave greatest seedling growth from large and medium seeds whereas 20 mm was more suitable for small seeds. Sowing depth is the mean vertical distance (cm) of seeds below the soil surface, after the seedbed has settled (Sylvester-Bradley and Roebuck 1985). The greatest seedling growth of small seeds was obtained when seeds were sown near the soil surface and this may reflect a general survival strategy adopted by *A. saligna* growth and suggests that small seeds should be sown at 2.5 cm or less. Sowing depth influences the aeration as well as penetration of water and light, which influences the yield through its effect on date of emergence and percent of plant emergence. Sowing deeper than necessary exposes the seeds to possible anaerobic conditions during wet periods and inevitably extends the pre-emergence growth period (Perry 1984). Evans (1984) suggested that deeper sowing increases root anchorage as well as moisture availability.

8.1.3 Growth under salinity

Due to the problem of salinity worldwide efforts are being made to combat it. One strategy in dealing with salinity had been suggested to be the growing of salt tolerant plants and has increased the need to understand salt tolerance in plants. *A saligna* adapts well to diverse environments. The approach of this study was to focus the responses of *A. saligna* to salinity in Libya in order to assess salt tolerance in this species.

Salinity stress of a plant is a combination of water stress (osmotic) and ion imbalance. Salt stress arises from excessive uptake of salts by plants and is a specific and

unavoidable consequence of high ion concentration, while ion imbalance results from altered ionic ratio in the cells after accumulation of the dominant (mostly Na^+ and Cl^-) salt which is responsible for the salinity of the medium. Survival in the greenhouse and field was significantly reduced by salinity. Frequency of NaCl irrigation had a significant influence on plant survival. Survival was not affected by one irrigation with 0.5 M NaCl, but increased frequency of irrigation reduced plant survival with irrigations of 0.5 M NaCl. However, irrigation with 1.0 M NaCl resulted in the mortality of all plants in greenhouse and field experiments when applied just once.

Increasing NaCl concentrations reduced growth of *A. saligna* in the field when irrigated with 1.0 M NaCl. This result agrees with Ansari, Khazada, and Azmi, (1988) who reported a negative response with 1.0 M and 1.5 M NaCl irrigation in pot trials seedlings of *Acacia stenophylla*, *A. ampliceps*, *A. auriculiformis*, *A. maconchieana A. bivenosa*, and *A. saligna.*

Establishment of *A. saligna* does not depend only on the concentration of NaCl, but also on frequency of irrigation. The plant survival with more than one irrigation decreased with increasing NaCl concentration. Aswathappa, Marcar, and Thomson (1987) compared the salinity tolerance of thirty seven species of *Acacia* in an experiment with 2 month-old seedlings treated in stepwise increments of 25 mM every 2 days and found differences among species in their response towards salinity. Several investigations have reported reduction in growth of plants as a result of salinity stress, e.g. in cotton (Meloni *et al*. 2001) and sugar beet (Ghoulam, Fares 2001). There were large interspecific differences although there was no consistent pattern in the response of different parameters to NaCl.

The fresh weight decreased with increasing NaCl concentration after seven weeks. Significant differences in fresh and dry weight were obtained between the plants treated with the lower NaCl (irrigated twice and three times with 0.5 M NaCl) and those with the higher NaCl (once, twice and three times irrigated with 1.0 M NaCl). However, irrigation with either 0.5 M or 1.0 M resulted in a marked decreased in the fresh weight per plant. The decrease in growth probably results from the decreased availability of water and increased toxicity of NaCl in the rhizosphere, produced by increased salinity of the water. This finding is similar to that found by El-Lakany and Luard (1986) in *Acacia* spp. in a greenhouse. They found also that the lower concentrations produced some growth stimulation in the most tolerant *Acacia*. Frequency of saline water irrigation affects biomass of plants. Increasing frequency of irrigation with saline water with either 0.5 M or 1.0 M NaCl decreased plant growth substantially. However, the poorest performance was obtained in the case of treatments irrigated three times with 1.0 M. NaCl.

Concentration and frequency of irrigation whether with 0.5 M or 1.0 M NaCl influenced root fresh and dry weight of plants. However, when 0.5 M level was used it produced more root fresh and dry weight then 1.0 M NaCl in both the greenhouse and field in Libya. Seemingly, irrigation with 1.0 M NaCl was harmful for *A. saligna* growing in a greenhouse, but the most severe effect was found in the field in Tripoli, Libya. This could be ascribed to the environmental interactions such as relative humidity, temperature, radiation and air pollution as studied by Shannon, Grieve, and Francois (1994). This result agrees with Aziz and Khan (2001) who found that the optimum growth of *mangrove* plants was obtained at 50% seawater and declined with further increased in salinity, while in *Alhogi pseudoalhagi* total plant weight increased at low salinity (50 mM NaCl), but decreased at high salinity (100 and 200 mM NaCl), but leaf

number was less affected. Neumann (1997) considered that inhibition of leaf growth by salt decreases the volume of new leaf tissues into which excess salt can be accumulated. Ghoulam, Foursy, and Fares (2002) and Fisarakis, Chartzoulakis, and Stavrakas (2001) found that sultana vines displayed a greater decrease in accumulation of dry matter in shoots than in roots, particularly attributed to high NaCl concentration. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by the roots.

The plant height, decreased markedly with increased concentration and amount of NaCl, However, salinity decreased the plant height within the increased NaCl irrigation. Irrigation with 0.5 M NaCl did not have any significant effect on plant height. Plant height was reduced with 0.5 M applied twice and three times and 1.0 M NaCl irrigation, the reduction in plant height was pronounced with 1.0 M NaCl irrigation, especially those irrigated three times, as compared with 0.5 M NaCl or non NaCl (control).

The effect of salt stress on the number of leaves was similar to that on plant height. Irrigation with 0.5 M NaCl once did not have any significant effect. Number of leaves was negatively affected with increasing amount and frequency of NaCl irrigation with NaCl at 0.5 M or 1.0 M two and three times. Neumann (1997) considered that inhibition of leaf growth by salt decreases the volume of new leaf tissues.

Moisture content of *A. saligna* increased with the first application of salt and progressively decreased to the lowest value with three applications of salt. This decrease in water content by salinity could be attributed to low ion accumulation in the shoot tissue and to osmotic balance by reducing the tissue water (Gulzar, Ajmal Khan, and Ungar 2005). Salinity can affect plant growth by reducing the amount of water available to the plant.

Salinity affects plant physiology through changes of water and ionic status in the cells According to Dubey (1997) and Yeo (1998), salt causes both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects. The general response of plants to salinity is a reduction in growth (Romero-Aranda, Soria, and Cuartero 2001, Ghoulam, Foursy, and Fares 2002). The initial and primary effect of salinity, especially at low to moderate concentration, might be attributed to its osmotic effects (Munns and Termaat 1986, Jacoby 1994). Osmotic effects of salts on plants result from reduced water potential due to increasing solute concentration in the rhizosphere. At very low soil water potentials, this condition interferes with the plant's ability to extract water from the soil and maintain turgor.

At high salinity, some specific symptoms of plant damage may be recognized such as necrosis and leaf tip burn due to Na^+ or Cl^- ions (Wahome, Jesch, and Grittner 2001). High ionic concentration may disturb membrane integrity and function; interfere with internal solute balance and nutrient uptake, causing nutritional deficiency symptoms similar to those that occur in the absence of salinity (Grattan and Grieve 1999). Ion imbalance especially results from altered ionic ratio in the cells after accumulation of the dominant (mostly Na^+ and Cl^-) ions of the salt which is responsible for the salinity of the medium. Irrigation with different concentration of NaCl resulted in significant accumulation of Na and decrease in K^+, Ca^{++}, Mg^{++}, P and Fe^{++} ion uptake. High salt (NaCl) uptake competes with the uptake of other nutrient ions, such as K^+, Ca^{++}, N^+, P (Perez-Alfocea, *et al.* 1996; Grattan and Grieve 1999, Bayuelo-Jimenez, Debouck, and Lynch 2003). The plants studied both in the greenhouse and in the field in Tripoli, Libya showed a significant increase in the amount of Na^+ in plants with increase in salinity of the external medium. Plants may respond to salinity by absorbing sodium at high rates and accumulating these ions in their leaves for osmotic adjustments to the low water

potential in the soil. Increase in Na^+ content of *A. saligna* cold be ascribed to high Na^+ content of irrigation water. This result agrees with that of Ghoulam, and Fares, (2001) who observed an increase in the Na^+ and Cl^- content in the leaves and roots of sugar beet with increasing NaCl concentration in the rhizosphere.

The amount of K^+ in the medium showed an increase with increase in external salinity, but there was a significant decrease of K^+ with an increase in irrigation water salinity in the plants with 0.5 M or 1.0M NaCl. The significant decrease in K^+ content of plants might be due to the phenomenon between Na^+ and K^+. Ca^{++} content decreased significantly with the increase in salinity. The Ca^{++} decreased with 0.5 M and 1.0 M NaCl in the field, but increased in the greenhouse experiments possibly due to high temperature and high evaporation. The significant increase in Na^+ content in the soil could be attributed to plants absorbing calcium from soil particles. Reduction in K^+ and Ca^{++} varied at different salinity levels. Mg^{++} also showed a significant decrease in plants in the field and in the greenhouse with the increased salt concentration of the irrigation medium.

Salinity stress has both stimulatory and inhibitory effects on the uptake of some micronutrients by plants (Grattan and Grieve 1999). Decrease of Mg^{++} content of leaves has also been reported upon salt accumulation in wheat. The deficiency of K^+, Ca^{++}, N and P reduced the growth and yield (Perez-Alfocea, *et al*. 1996, Grattan and Grieve 1999, Khan, Ungar, and Showalter 2000, Bayuelo-Jimenez *et al*. 2003). Salinity affected phosphorus content resulting in a significant decrease in its concentration in plants in the field and in the greenhouse experiments. Ansari (1990) found that the salinity may increase or have no effect on P uptake, while salinity decreases the concentration of P (Sharpley, Meisinger, and Suarez 1992).

8.1.4 Growth with Zander

Zander can play a significant role in soil fertility. Zander has important physical and chemical properties and application of Zander improved soil fertility. The magnitude and stability of beneficial changes depends on the amount of Zander applied. Soil texture governs the moisture and nutrient storage capacity, permeability and water holding capacity of soil (Brady and Weil 1999). Zander amendment may improve seedling growth and establishment. The addition of 10% or 30% Zander increased the moisture content at field capacity and thus the amount of plant available water in soil compared with the soil without Zander.

The positive effects of Zander application on plant survival as well as on other growth parameters of *A. saligna* seedlings were evident. There were significant interactions between Zander level, NaCl concentration and frequency of irrigation with salinized water. The interaction between Zander and salinity levels was also significant. All growth parameters decreased with 1.0 M NaCl irrigation, yet Zander application led plants to persist when it comprised 30% of soil media.

The positive impacts of Zander on survival and plant growth can be attributed to its chemical and physical properties, since it provides the nutritional elements such as N, P and K^+ as well as increasing higher water holding capacity, thereby alleviating the hazardous impacts of salinity (Zhu and Chen 1994, Su *et al*. 2003). The partially decomposed residue is called effective humus which represented about 40% of the total weight (Zander Corporation 2007). Zander organic matter improved soil structure and led to a change in soil texture.

The high ionic concentration in the cell is responsible for most of the biochemical changes which affect the cellular metabolic level, and consequently the growth and development of the plant. The concentration of elements absorbed by plants was higher in plants grown in 30% Zander. Brown *et al*. (1987) found that compost increases the crop growth rate by an increase in leaf area and also by an increase in radiation use efficiency. Potassium has a significant role in many important physiological processes in the plants (Schwartzkopf 1972). Phosphorus is an important nutrient for all crops. Crops need phosphorus for their growth and for their metabolic reaction (Ahmed *et al*. 2003). Phosphorus is usually found in plants at very low concentrations, and is therefore considered as a limiting factor for plant growth.

The highest shoot dry weight was obtained in unsalinized plants grown in the medium with 30% Zander or 10% Zander. The lowest shoot dry weight was obtained with three applications of 50 ml 0.5 M NaCl or 1.0 M with plants grown in a medium with 0% Zander. The decrease in shoot dry matter with salinity of plants grown in medium with no Zander (Control) or low level of Zander might be ascribed to limited supply of metabolites to young growing tissues (Mass and Nieman 1978) or interference of NaCl with the production of proteins or damage to enzyme proteins exposed, to low water potential. The highest dry weight obtained was detected in the plants grown in a 30% Zander medium. The positive effect of Zander on dry weight even under salinization could be ascribed to the presence of sufficient nutrient elements, since the higher the Zander level, the higher the dry weight obtained. In addition, retention of water by aid of Zander may be helpful in minimizing the hazardous impact of salinity. High salinity caused a reduction in the moisture content, so that the growth of plants was reduced. Glenn (1987) reported that water content of 19 grasses declined with an increase in salinity. Water moisture in *A. saligna* increased with the first application of salt and

progressively decreased to the lowest value after six applications of salt. Gulzar, Ajmal Khan, and Ungar (2005) attributed the decrease in water content by salinity to the low ion accumulation in the shoot tissue and to osmotic in balance by reducing the tissue water. On the other hand, the concentration of Zander significantly affected moisture content of *A. saligna* owing to its protective effects toward water losses from the soil.

At high concentrations of salt, the external osmotic potential may be depressed below that of the cell water potential resulting in osmotic desiccation. This increase in sodium concentration was accompanied by a progressive decrease of potassium concentration. Epstein (1961) showed that there is an antagonistic relationship between K^+ and Na^+ uptake and such antagonism may be due to the direct competition between K and Na at a site of ion uptake in the plasma lemma of feeder root cells. Substantial differences in Na^+ and K^+ accumulation between salt-resistant species may be due to differences in ion selective transport capacity at root level (Wang *et al*. 2002). Glenn (1987) in his study on 14 grasses measured ash and cations and found that in response to salt stress, Na^+ in shoots increased, K^+ decreased and water content decreased. He suggested that grasses maintain osmotic balance by water loss rather than sodium uptake.

The average calcium concentrations for 10 and 30% of Zander were approximately 2 fold that of 0% Zander. The ameliorative effect of Ca^{++} on Na^+ toxicity has been reported (Epstein 1961) and Viets (1944) stated that a certain level of calcium is required for maximal uptake of nutrient ions. Calcium acts presumably by maintaining the membrane with its proteins and lipids in a proper physico-chemical state (Rengasamy 1987). There are several reports suggesting a close connection between membrane integrity and calcium, and the consequent effects on ion transport. Supplemental Ca^{++} can affect the length of the growth zones of salt-stressed plants. In

sorghum leaves, the length of the growth zone is shortened with 100 mM of NaCl salinity. There are several reports supporting the involvement of Ca^{++} signalling in salt tolerance. Ca-binding protein is induced in salt-stressed *Arabidopsis* (Jang *et al.* 1998). It was also found that after salinization, there are substantial increases in mRNA levels of a Ca-ATPase in tomato (Wimmers, Ewing, and Bennett 1992).

Phosphorus uptake by plants grown in a medium with 30% of Zander was as much as three times higher than in plants grown in Zander-free soil and twice that with 10% Zander. In most cases, salinity decreases the concentration of P in plant tissue (Sharpley, Meisinger, and Suarez 1992), but the results of some studies indicated that salinity either increased or had no effect on P uptake. Champagnol (1979) concluded that the addition of P to saline soils increased growth and yields in 34 of the 37 crops studied and stated that added P had no effect on, or decreased salt tolerance as salinity increased from low, to moderate, to high levels, respectively. Ca^{++} content in soil varied by salinity and by Zander concentration. Frequency of irrigation with 0.5 M or 1.0 M NaCl may affect the Ca^{++} status of the soil due to changes in its solubility. In non-saline sodic soils, increase in exchangeable sodium is balanced by a decrease in exchangeable Ca^{++} and Mg^{++}, leading to Ca^{++} and/or Mg++ deficiencies in plants when these ions are deficient in soil solutions (Rengasamy *et al*. 1984).

The availability of micronutrients might be reduced in saline soils owing to pH increase. Maintenance of adequate levels of K^+ is essential for plant survival in saline habitats. The selectivity of the root system for K^+ over Na^+ (Grattan and Maas 1985) together with adequate levels of K^+ provided by Zander might improve K^+ uptake by plants. Phosphate availability is reduced in plants after irrigation with saline water because of ionic strength effects that reduce the activity of phosphate but also because phosphate

concentration in soil solution is tightly controlled by sorption process and by the low-solubility of Ca-P minerals. Phosphorus concentration in the plant tissue declines with the increase of NaCl (Sharpley, Meisinger, and Suarez 1992). Therefore, it is understandable that phosphate concentrations in field-grown agronomic crops decreased as salinity increased.

Analysis of the shoots of surviving plants showed that Na^+ concentration in the shoots increased with increasing concentration and number of applications of NaCl. However, Zander did not prevent the uptake of Na^+ by *A. saligna* and surviving plants growing in 30% Zander had a significantly higher Na^+ content than those growing in sand and soil a lone at most levels of NaCl stress, suggesting that exclusion mechanisms are probably not involved. Ca^{++} concentration in the shoots of plants was decreased by NaCl treatment and increased by Zander amendment such that the Ca^{++} concentration in plants grown in 30% Zander and exposed to 1.0 M NaCl was three times higher than in unstressed plants grown in sand alone.

8.1.5 Mechanism

Two hypotheses were that Zander improves salinity tolerance by (1) improving water holding capacity and preventing drought effects and (2) increasing uptake of Ca^{++}. The two hypotheses are not mutually exclusive and are unlikely to be the only beneficial properties of Zander. The effect of water holding capacity in the 30% Zander and extra water applied to *A. saligna* grown with 0% or 10% Zander was investigated in Chapter 7. Extra water improved survival, to the same level as 30% Zander and no extra water. However, the greatest positive effect was from survival with 0%, 10% Zander when irrigation with 1.0 M NaCl and extra water applied compared with control irrigation

with 1.0 M NaCl without extra water. Extra water applied had a significant effect on stem height but not to the same level as with 30% Zander. However extra water also increased number of leaves, leaf area shoot fresh and dry weight to the same level as 30% Zander with saline irrigation, Moreover there was no significant effect on leaf area without saline stress, and root fresh weight with 10% Zander.

Extra water supplied to pots with 0% or 10% Zander increased Ca^{++} and K^{+} to the same level as 30% Zander when irrigated with saline water. In the absence of salinity stress, addition of extra water to pots with 0% Zander, and with salinity stress addition of extra water to 10% Zander, reduced Na^{+} absorption by *A. saligna*. Addition of extra water to pots with 0% and 10% Zander increased the Ca^{++}/Na^{+} and K^{+}/Na^{+} ratios in the plant. Thus the beneficial effects of additional water may be direct by reducing the osmotic stress of salinity, or indirectly by altering the ionic balance of the plants.

One possible approach to reduce the effect of salinity on plant productivity is through the addition of calcium. Several reports have indicated that supplement Ca^{++} may alleviate the reduced growth caused by NaCl salinity. The effect of Ca^{++} on *A.saligna* was investigated in Chapter 7. Both 0% and 10% Zander and extra Ca^{++} applied was effective in partly amelioration salinity stress effects on growth and mineral uptake. The positive effect of Ca^{++} addition to soil alone (0% Zander) may be due to less Na^{+} in the plant this protects cell membranes from the adverse effect of salinity (Busch 1995). Zander with 0% and 10% and extra Ca^{++} applied led to statistical increases in the plant survival of *A. saligna* and also plant height and leaf number, but did not increase fresh and dry weight of shoot, root and moisture content. Supplementary Ca^{++} ameliorated the negative effects of salinity on these parameters. Zander and extra Ca^{++} with 0% and 10%

Zander did not statistically increase shoot and root fresh and dry weight to the same level as 30% Zander and no extra Ca^{++}.

The increase in survival, shoot height, leaf number and leaf area might be due to improvement of the structure of the soil by increasing the soil nutrition which significantly increased with 30% Zander and 10% Zander compared with soil alone. Soil amended with Zander had good aeration and drainage that encourages better root growth, and nutrient absorption, enhanced the availability of certain elements and their supply to the plant during the growth period. Indeed Zander increased P, K^{+} and Mg^{++} in the plant as well as Ca^{++}.

There is a significant difference between Zander and Zander with extra Ca^{++} with respect to K, Mg, and P concentration. However, the concentrations of the elements in plants grown in medium of 30% Zander were higher than those in medium of 10% Zander + extra Ca^{++} under salinized condition. The role of Ca^{++} with growing medium increase the survival and growth of *A. saligna* plants exposed to NaCl stress. Supplement calcium increased the Ca^{++}/Na^{+} and K^{++}/Na^{+} ratios.

8.2 Conclusions

The results confirm that *A. saligna* seeds have hard seed coat dormancy which already known can be broken by mechanical or chemical scarification or soaking in hot water. Mechanical scarification by cutting the seed coat gave the highest germination percentage. Mechanically scarified seeds imbibed water and germinated to high percentages, whereas unscarified seeds did not take up water.

Mechanical scarification was the most effective for *A. saligna*, but cannot used to treat a large amount of seeds. Therefore soaking seeds in boiling water three times before

sowing appears to be the best method suited to large quantities of seeds of *A. saligna* and is regarded as easy and safe in practice.

Large seeds not only displayed germination earlier than small ones, but also displayed greater germination level. Sowing of *A. saligna* seeds at 30-20 mm depth generally gave the greatest seedling growth from large and medium seeds, whereas 20 mm was more suitable for small seeds.

Moderate salinity levels retarded growth and reduced yield, while high levels killed *A. saligna*. Irrigation with NaCl affects plant growth by reducing the amount of water usable to the *A. saligna* seedling and by increasing the concentration of certain ions that have a toxic effect on plant metabolism. Irrigation with NaCl significantly decreased plant survival and emergence at 0.5 M or 1.0 M NaCl.

Survival of seedlings was greater with 0.5 M than with 1.0 M NaCl. The biomass of *A. saligna* growing either in a greenhouse or in the field was negatively affected by the saline irrigation, especially with 1.0 M NaCl.

The addition of Zander at the rate of 30% to the soil induced greater growth and salinity resistance of *A. saligna* plants even at a salinity level of 1.0 M NaCl. If it is required, irrigation once with saline water at the seedling stage may give a better result than two or three irrigations, due to less ion accumulation and toxicity effects.

Zander increased K^+ that was reported that might serve as an indicator of crop salt tolerance and provided high amount of Ca^{++} whose ameliorative effect on Na^+ toxicity has been reported. Zander improved plant survival and growth in the absence of salinity and ameliorated the effect of salinity by increasing water holding capacity and preventing drought effects. Extra water supped to the plants increases survival, growth

even in the absence of Zander. The beneficial effect of Zander is not due to reduction in Na^+ uptake by the plants as plants grown with Zander take up more Na^+ than plants grown in sand or soil. With extra water Zander increases uptake of Ca^+ by *A. saligna* and Ca^{++}/Na^+ and K^+/Na^+ ratios. The beneficial effect of Zander on salt tolerance can be partly reproduced by supplying extra water in the absence of the organic material itself.

The beneficial effects of Zander on survival and plant growth were reproduced by increasing the Ca^{++} concentration in the plants to the level found with Zander by the application of Ca^{++}. Supplemented calcium increased the Ca^{++}/Na^+ and K^{++}/Na^+ ratios in shoots and improved plant survival and growth. Increasing external Ca^{++} reduced Na^+ uptake with soil alone (no Zander) in *A. saligna* and thus may be an important factor in controlling salinity effects.

8.3 Recommendations

- For mass production of *A. saligna* seedlings in nurseries, it is advisable to pre- treat seeds to overcome had seed coat (dormancy) before sowing in medium to achieve rapid germination and increase germination level. However, it is recommended to soak the seeds in boiling water as a safe and cheap method rather than using other methods. Soaking of seeds in concentrated acids can be used, but it is not safe and is expensive compared with soaking in boiling water.

- Selection of large (length ≥ 8 mm, width ≥ 3 mm) sized seeds rather than medium (length < 8 mm and ≥ 5 mm, width ≥ 2.5 mm), and small (length < 5 mm and ≥ 3 mm, width ≥ 2 mm). ones is recommended, since they contain sufficient stored food in endosperm essential in the critical period or germination. In addition, the large-

sized seed contained a bigger embryo which can grow under hazard condition as compared with small and medium-sized one.

- Depth of seed sowing is one of the most important factors that can play a role in germination success. Sowing of seeds 2.5-3 cm deep under soil surface in the nurseries is recommended to achieve rapid germination and shorten the time needed for establishment of the seedling.

- If there is no usable fresh water temporarily in a nursery, irrigation with saline water can be recommended to irrigate seedlings of *A. saligna*, but with special care, including use of low concentration of salt (not more than 0.5 M NaCl) and not more than twice or three times according to prevailing conditions.

- The organic matter "Zander" is regarded as a promising additive which can be mixed with the soil or sand of seedlings to improve its chemical and physical properties.

- The application of Zander also enhanced water and salt holding capacity of soil. Increased concentration of Ca^{++}, Mg^{++}, Na^{+} and K^{+}. It is recommended to add Zander by 30% to the soil to improve water holding capacity to alleviate evaporation on the one hand and to aid seedlings of *A. saligna* to resist salinity on the other hand.

- Increasing the supply of water to the plants increases survival, growth and salinity tolerance even in the absence of Zander. Use of organic matter amendments to enable arid-zone plants to resist salinity merits further research not only for the seedling stage, but for subsequent ones as well. In temperate climates where *A.*

saligna is direct seeded, growth may be increased by increasing Ca^{++} and H_2O supply.

- It is not recommended to add calcium to soil amended with Zander, since the latter has an adequate amount of the element. The possible improvement of plant growth by Zander in the present study may be due to improved water holding capacity and availability of plant nutrients, especially Ca^{++}.

8.4 Future work

Future field studies could include application of high Zander concentration in tree nurseries or in the field in the coastal area in Libya where good quality water is not available. The main requirement in the field will be analysis of soil and plant samples and a detailed investigation of chemical and physical properties of Zander in mixture with soil in order to explain the interaction between the Zander and saline water.

Further studies are required to determine the effect of Zander on salt tolerance of other *Acacia* spp. such as *A. salicina, A. senegal, A. cyclop* and *A. nilotica* which also grown successful in Libya.

More information on the effect of salinization and Zander on productivity of land and the economic implications of the amelioration measures are necessary. The use of other organic amendments such as compost that might be cheaper and more readily available than Zander and it is recommended that chemical benefit analysis is carried out.

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Appendix 1: Environmental data of Libya

Libya meteorology department
Climatologically section
Station name and No Tripoli city

Table1.1: Mean temperature (ºC) in Tripoli from (2007-2009)

Year	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
2007	14.3	14.6	15.6	19.5	22.2	28.6	26.7	29.5	27.3	23.7	18.0	13.8
2008	13.4	13.1	16.7	20.4	24.8	25.6	29.3	28.0	27.7	24.2	18.5	13.5
2009	14.2	13.6	16.2	22.2	22.9	26.5	29.1	29.3	27.5	23.4	18.3	13.6

Table 1.2: Mean relative humidity % in Tripoli from (2007-2009)

Year	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
2007	79	75	71	70	63	57	68	68	68	68	60	74
2008	81	75	63	56	57	66	62	68	59	67	64	73
2009	73	60	60	59	61	59	61	66	65	68	61	69

Table 1.3: Mean wind sped (km/ h) Tripoli from (2007-2009

Year	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
2007	4.3	6.3	5.7	8.2	6.6	6.3	4.7	5.1	5.3	5.7	3.6	5.1
2008	4.4	3.8	5.8	6.1	8.5	6.7	4.6	5.0	5.0	5.5	4.3	5.4
2009	6.0	6.9	7.1	8.5	7.8	7.1	5.1	4.6	5.1	5.4	3.9	5.3

Table 1.4: Total rainfall (mm) in Tripoli from (2007-2009)

Year	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
2007	12.6	50.6	43.2	7.7	0.0	0.0	0.0	0.0	0.0	90.5	35.2	75.5
2008	137.6	69.9	1.3	0.6	1.0	0.5	0.0	1.5	6.0	1.60	29.6	80.5
2009	29.6	15.7	0.1	0.1	0.0	0.0	0.0	0.1	1.4	1.20	30.8	71.9