SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

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Effect of Saline Water on Germination and Vegetative Growth of Faba Bean (*Vicia faba* L.)

تأثير الماء المالح على الانبات والنمو الخضري للفول المصري

(*Vicia faba* L.)

A Dissertation Submitted in Partial Fulfillment of the Requirements of the Degree of Master of Science in Horticulture

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الآية

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

قَالَ تَعَالَىٰ:

﴿ وَهُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً فَأَخْرَجْنَا بِهِۦ نَبَاتَ كُلِّ شَىْءٍ فَأَخْرَجْنَا
مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ ٱلنَّخْلِ مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ
وَجَنَّٰتٍ مِّنْ أَعْنَابٍ وَٱلزَّيْتُونَ وَٱلرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَٰبِهٍ ۗ ٱنظُرُوٓا۟ إِلَىٰ ثَمَرِهِۦٓ
إِذَآ أَثْمَرَ وَيَنْعِهِۦٓ ۚ إِنَّ فِى ذَٰلِكُمْ لَءَايَٰتٍ لِّقَوْمٍ يُؤْمِنُونَ ﴿٩٩﴾ ﴾

صدق الله العظيم

سورة الأنعام الآية(99)

DEDICATION

To My dear mother

To my husband

To my sister

To my teachers

To my colleagues

And my friends

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Thanks giving before and after to God Almighty.

I would like to thank my supervisor Mohamed Osman Warrag,who lend a helping hand to me in my research, and also extend my sincere thanks to my teachers, in the Department of Horticulture . My deep thanks and gratitude are extended to Dr. Gafar Ali Farah, Department of Agronomy. Great gratitudes and thanks are extended to Dr. Zaheereldin Abdalla Ali.

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Contents

Abstract

This study was carried out to investigate the effect of different concentrations of sodium chloride (NaCl) solution on the germination and the vegetative growth of faba bean (*Vicia faba* L.) cv Agabat. The NaCl solution concentrations used were 0.00 (distilled water as a control), 0.032, 0.063, 0.125, and 0.250 molar. The daily and final germination percentage was not significant affected by the 0.00, 0.032 and 0.063and 0.125 M. The final germination percentage for these NaCl concentrations were above 90%, whereas that for the 0.250 M was significantly less (70%) and no seeds had germinated using the 0.50 M NaCl solution. The rate of germination, as judged by time to 50% of the final germination percentage (GT50), the germination rate index (GRI) and the corrected germination rate index (CGRI) was significantly higher for the distilled water in comparison with the NaCl solutions tested. In contrast the 0.250 M NaCl solution resulted in the lowest seed germination rate. The other solutions resulted in almost similar values. The weekly height of the plant was not very much affected by the solutions with the exception of the 0.125 M which killed the plants at the fourth and fifth weeks. The same trend was followed by the number of leaves per plant. The fresh and dry weights of plants are the same for the solutions with the exception of the 0.125 M NaCl solution which resulted in statistically significant lower weights. From this study it seems that more research is needed to investigate the response of this popular legume in the Sudanese diet to the saline irrigation water due to the exploitation of upper lands which are irrigated with waters from wells, most of which were saline to some extent.

الخلاصه

أجريت هذه الدراسه لدراسة تأثير تراكيز مختلفه من محلول كلوريد الصوديوم على الانبات والنموالخضري لمحصول الفول المصري صنف (عقبات). تركيزات المحلول التي استخدمت هي 0.00 (ماء مقطر-الشاهد)، 0.032،0.063، 0.125 و0.25 مولار. نسبة الانبات اليوميه والنهائيه لم تتأثر تأثيرا معنويا باضافة التراكيز 0.00،0.032،0.063،0.125 مولار. النسبه النهائيه للانبات لكل تراكيز كلوريد الصوديوم كانت أكثر من 90%عدا التركيز0.25 الذي أعطى 70% ولايوجد انبات عند اضافة 0.50.مولار من محلول كلوريد الصوديوم.

تم تقييم معدل الإنبات باستخدام الوقت اللازم للوصول إلى 50% من نسبة الإنبات النهائيه (GT50) ومؤشر معدل الانبات (GRI) ومعدل الانبات المعدل(CGRI). كان معدل الانبات مرتفع معنويا في الماء المقطر مقارنة بمعاملات محلول كلوريد الصوديوم المختبره. بالمقابل سجل التركيز 0.25 مول كلوريد الصوديوم أقل معدل انبات للبذور.بقية التراكيز سجلت في كل الحالات قيم متساويه تقريبا. لم تتأثرقراءات طول النبات الاسبوعيه كثيرا باضافة التراكيز ماعدا التركيز 0.125 مولار اذ أدى الى موت النباتات في الاسبوع الرابع والخامس،نفس الحالة انطبقت على عدد الاوراق في النبات. كل التراكيز للمحلول كانت متساويه في الوزن الرطب والوزن الجاف عدا التراكيز 0.125 مولار كلوريد الصوديوم والذي أعطى إحصائيا أقل وزن. تشير هذه الدراسه الي أهمية إستمرار الأبحاث لدراسة تأثير الملوحه على أفضل محصول بقولي لغذاء السودانين وذلك لوجود مساحات شاسعه من الأراضي التي تروي بمياه الابار المالحه.

CHAPTER ONE
INTRODUCTION

Faba bean *(Vicia faba* L.) belongs to the family Fabaceae (Leguminosae). It is believed to be originated in the Mediterranean region or in the west or central Asia.

Faba bean is one of the most important legume crops worldwide, offering high nutritive value, capable of returning atmospheric nitrogen in association with *Rhizobium* species to the soil. The feeding value of faba bean is high and this legume has been considered the most extender or substitute due to its high protein content (20.4%) (Javaid, 2010). Most of the people depends on faba bean in their daily food, this mainly due to the fact that it represents a cheap source of protein compared to animal source and also used as animal feed.

The production in the world is concentrated in nine major groecological regions, namely, Northern Europe, Mediterranean, the Nile Valley, Ethiopia, Central Asia, East Asia, Oceana, Latin America and North America. According to the statistics of the food and Agriculture Organization (FAO, 2000), the total area under faba bean in the world was about 3227 million hectares, producing 3.83 metric tons. It is an important food legume crop in the Sudan, it is concentrated in the Northern State, north of latitude 16^0, where environmental conditions are suitable for its production better than in other parts of the country. In this state, more than 70% of this crop is produced. The Nile State ranks second and produces about 20%.Small amounts are produced in Khartoum State. The crop is also an important source of income for farmers in Northern Sudan. It contributes to soil fertility through biological nitrogen fixation (Ageeb,1995). The annual production of faba bean in the Sudan is estimated to be 143000 metric tons (FAO, 2002). Faba bean production and marketing in River Nile State is facing many constraints. Ahmed

(1995) summarized the major constraints to production of faba bean as follows: (a) limited cultivable land, (b) poor management practices, (c) low yielding traditional cultivars, (d) insect pest and diseases and inadequate credit and marketing**.**

Salinity stress is a limitation to the productivity of agricultural crops worldwide. It has been estimated that almost 80 million hectares of arable lands worldwide is currently affected by salinity (FAO, 2008). While improvements in land and water management can alleviate the problem, improving the tolerance of crop species to salinity will also contribute to increases in productivity. The soil solution of saline soils is composed of a range of dissolved salts, such as NaCl, Na_2So_4, $MgSo_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2Co_3, each of which contribute to salinity stress, but NaCl is the most prevalent salt and has been the focus of much of the work on salinity to date (Rengasamy, 2002; Munns and Tester, 2008).

The most common plant response to salt stress is a general reduction in growth and yield. When salt concentration increases above a threshold level, both the growth rate and ultimate size of crop plants progressively decrease (Neumann, 1997; Aly *et al*., 2003). Growth suppression seems to be a nonspecific salt effect that is directly related to the total concentration of soluble salts or osmotic potential of the soil water. Within limits, concentrations of different combinations of salts cause nearly equal reductions in growth. It was found that about 250 thousand hectares in the Northern Sudan were affected to some degree by sodicity or salinity (Ali and Fadl,1977). The largest areas are mostly found to the north of Khartoum along both banks of the main Nile. These areas are located in the arid zone with relatively cool winters which favor the growing of high value fruits and vegetables. Salt affected soils of the Sudan were often found in Khartoum province, along the main Nile, Gezira clay plain and the White Nile and Northern states.

Therefore this study was conducted to investigate the effect of different concentrations of sodium chloride solution on germination and vegetative growth of faba bean.

CHAPTER TWO

LITERATURE REVIEW

Faba bean is also commonly referred to as field, horse, tick and winds or bean, although all but the latter common names usually refer to cultivars not used as vegetables for human consumption. Faba bean probably originated in west or central Asia (Pilbeam and Hebblethwaite, 1994). It is widely cultivated in temperate areas and in the tropics, subtropics and arid areas as a cool season crop. This species is cultivated as stock feed (as mature seed, hay or silage) and as a vegetable. The vegetable cropt is either produced for the slightly immature seeds which are cooked fresh or processed. In some arid and tropical areas the dried mature seeds are stored for use in the winter and other seasons when fresh vegetables are in short supply. The origins, yield variability, breeding and prospects for the species have been discussed by Pilbeam and Hebbleth waite (1994). Earlier classifications of the range of cultivars used by agriculture and horticulture were based on their agronomic uses; however, as a result of the large degree of overlap between the different types this is no longer of value.

2.1. Botany:

Faba bean is an annual herb with coarse and upright stem, un-branched 0.3-2m tall, with one or more hollow stems from the base (Bond *et al*.,1985; Duke, 1981; Heath *et al*.,1994).The leaves are alternate, pinnate and consist of 2-6 leaflets each up to 8 cm long and unlike most other members of the genus, it is without tendrils or with rudimentary tendrils (Kay,1979;Bond *et al*.,1985). Flowers are large, White with dark purple marking borne on short pedicels in clusters of 1-5 on each auxiliary raceme usually between 5 and 10 nodes, 1-4 pods develop from each flower cluster, and growth is indeterminate though determinate mutants are available. About 30% of the plant in a population are cross-fertilized and the main insect pollinators are bumblebees. It bears a round paucobust taproot with profusely branched secondary roots (Bond *et al.,*1985). Based on seed size, two subspecies were recognized, pauijuga and faba. The latter was subdivided into var. minor with small rounded seeds (1 cm long), var. equine with medium sized seeds (1.5 cm) and var. major with large broad flat seeds (2.5 cm) (Kay,1979; Bond *et al*., 1985). Cubero (1974) suggested four subspecies namely: minor, equine, major and paucijuga. Taxonomically, the crop belongs to section faba of the genus Vicia (Bond *et al*., 1985; Smart, 1990). The majority of broad bean cultivars require long days for the acceleration of flower initiation, although this is not so with the earliest-flowering cultivars (Whyte, 1960). There is also evidence that vernalization at temperatures below 14 °C will also accelerate flowering. Faba beans are self-compatible, but both self- and cross-pollination occur. Insect activity is responsible for up to 30% crossing (Watts, 1980). Reisch (1952) found that high rainfall during flowering prevented pollination by insects and thereby was responsible for a considerable reduction in yield.

2.2. Ecology:

Faba bean requires a cool temperature for good development. The optimum temperature ranges from 7°C to 27°C (Duke, 1981). Faba bean is a long day plant and requires a cool season for best development and can be seeded early. Broad bean could be grown successfully on wide range of soils from heavy clays to light silty, the highest yield expected on loamy of 6-7pH (Kay, 1979). Rainfall of 560-1000mm per annum with a good distribution was reported as ideal (Kay, 1979). Bond *et al.* (1985) showed that the maturity period ranges 90-220 days depending upon the cultivars and the prevailing climatic condition.

2.3. Cultural Practices.

2.3.1. Seedbed preparation:

For best results, a good seedbed should be prepared, to insure good soil to seed contact. Since faba bean emerges slowly, time spent in preparing a good seedbed will help reduce problems with faba bean and with early weed control.

2.3.2. Seeding date:

Plant early if weather and soil conditions permit. Yields are reduced significantly when planting is delayed. Faba beans grow best under cool moist conditions; the seedlings are frost tolerant, but cannot tolerate heat during flowering. They are a legume and must be inoculated with specific inoculant to promote nitrogen fixation.

2.3.3 Method and rate of seeding:

Faba beans, which can be grown as a cultivated row crop or as a non-cultivated narrow-row crop like small grains, respond favorably to narrow row spacing. The optimum seeding rate was 160 kg/ha when planted in 20cm rows. Although high rates of sowing and narrow rows tend to produce higher yields, seed cost is an important restriction to optimum seeding rate. Planting depth is critical, since the hard, dry seed takes longer to absorb water and germinate than does common bean.

2.3.4. Irrigation:

Faba beans are very responsive to irrigation carried out during anthesis.Brouwer (1949, 1959) found that dry soil at this stage had the greatest adverse effect on yield, while irrigation before the onset of anthesis had relatively little advantage even under dry conditions. However, Jones (1963) found that seed yield was to some extent dependent on the plants having a relatively high growth rate before flower development. It is, therefore, considered preferable to ensure that the plants receive sufficient water during early development and also from the start of flowering.

2.3.5. Weed control:

Cultural and Mechanical: Faba beans are poor competitors with weeds, particularly in the seedling stage. This makes integrated weed control essential for successful crop production. Select fields with light weed pressure. The primary tillage several weeks before planting kills emerged weeds with shallow tillage just ahead of planting. Rotary hoeing fields 7 to 10 days after planting with a row cultivator is practiced if rows are 50 cm or more apart.

2.3.6. Harvesting and processing:

Signs indicating that a broad bean seed crop is ready for harvest include the pods becoming relatively dry with a loss of sponginess; this is preceded by a general blackening of the pods. The optimum seed moisture content at harvest is 16–20% . The crop is either cut by hand and tied in bundles or mown by machine (Raymond, 1985).

Bundling and stoking is necessary in northern Europe, although not in the drier seed production areas such as the Middle East and north Africa.

The material is threshed when dry at a drum speed of 250 rpm. Care must be taken to adjust the concave setting according to the seed size of the cultivar to minimize mechanical damage to the seeds (Raymond,1985).

2.3.7. Drying and storage:

Rapid drying at high temperatures often causes stress cracks. The maximum moisture content for a "straight grade" of faba beans is 16%. It is grown for human consumption and it is sometimes harvested early as green vegetable as in European countries or late as dry bean as in most of tropical countries.

2.4. Pests and Diseases:

Aphids may attack growing point (buds) in faba bean, controlled by malathion liquid spray, applied as soon as aphids observed (Tindall, 1968).

In wet soil conditions and with high temperatures root rots attack plants; improvement in drainage is essential if this occurs (Tindall, 1968).

2.5. Production of faba bean in Sudan:

Sudan depends mainly on local production and imports only very small amounts. Both acreage and production of faba bean have increased rapidly during the last twenty years, the crop area tripled and yield have increased and become more stable.

2.6. Faba bean fertilization:

Legumes require neutral to alkaline soil for maximum nitrogen (N) fixation by nodule bacteria. Soils should be tested and, if necessary, limed to at least pH 6.0. Dolomitic limestone would need to be applied at least one year prior to faba bean production. As a consequence of inappropriate applications of chemical fertilizers during continuous crop cultivation, many countries suffer from problems such as pollution of agricultural lands, water resources, and soil salinization. In an attempt to reduce these chemical inputs and raise soil quality as well as improve crop production, biotechnological practices such as application of biofertilizers have been investigated (Talaat and Abdallah, 2008). Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes. The nitrogen-fixing interaction between rhizobia and legumes and the mycorrhizal association between fungi and most of land plants are the most two commonly studied symbioses (Talaat and Abdallah, 2008). Compost is a highly diverse group of organic soil amendments which provides substantial nutritive fertility to soils. Dairy manure compost supplies not only the major nutrients (N, P, and K), but also a broad range of secondary nutrients, micronutrients and organic matter. Dairy manure can also improve water and nutrient holding capacity of the soil, reduce erosion, and reduce fluctuations in soil pH. Nutrients in compost products are more stable and are typically released gradually over three or more years; whereas inorganic fertilizers are generally formulated to release nutrients within a year of application (David *et al*., 2011).

Biological nitrogen fixation (BNF) can act as a renewable and environmentally sustainable source of N and can complement or replace fertilizer inputs. Its use can mitigate the need for fertilizer nitrogen, with concomitant benefits accruing in terms of effects on the global nitrogen cycle, global warming, and ground, and surface, water contamination. Intercropping legumes and other species capable of symbiotic N_2 fixation offer an economically attractive and ecologically sound means of reducing external inputs and improving the quality and quantity of internal resources. Nitrogen from this source (biologically fixed N_2) is used directly by the plant, and so is less susceptible to volatilization, denitrification and leaching. BNF is a kind of beneficial plant–microbe interaction that provides a restricted range of plants with the often-limiting macronutrient-nitrogen (Neera and Geetanjali, 2007).

Rhizobia stimulate plant growth mainly by modifying root development, which improved macro and micronutrients and water uptake, particularly in the early stages of plant development (Antoun *et al*., 1998). Most agricultural crops are colonized by arbuscular mycorrhizal fungi (AMF). In this symbiotic association, host plants provide the fungi with carbohydrates and in return receive mineral nutrient. AMF can enhance growth of crop plants through increasing nutrient uptake, particularly phosphorous (Ryan and Angus, 2003).

2.7. Salinity:

In many countries, maximizing the productivity of salt affected soils through fertilization is necessity to provide adequate food for the present population. Crop yield decline with salinity was reported by many research workers, but for a given level of salinity there was an increase in yield with fertilizer application (Amer, 1964; Lunin, 1965). This decrease in yield with salinity was attributed to reduction in the availability of plant nutrients under saline condition (Lucken, 1962).

Sameni *et al*. (1980) found that dry bean weight and its nitrogen content increased with increasing rate of applied nitrogen fertilizer. They also reported reduction in growth and nitrogen content when plants were irrigated with high saline water at all nitrogen levels tested, but the uptake of chloride and sodium was increased. However, it was postulated that the suppressing effects of high salinity were partially alleviated by nitrogen fertilization. An increase in plant salt tolerance with nitrogen fertilization has also been reported by Revikovich and Porath (1967) and Bernstein *et al*. (1974) using corn, tomato and wheat.

Champagonal (1979) stated that the increase in yield usually observed when phosphorous was applied to crop grown under saline conditions, did not of itself demonstrate the existence of phosphorous and salinity interaction, nor did it indicate the sign of this interaction as described by Bernstein (*et al*., 1974). However, phosphorous application to saline soils resulted in yield increase than most situations. This useful effect generally takes the form, at moderate salinity levels, of a ppositive phosphorous and salinity interaction.

2.8. Salt in the soil of Sudan:

It was found that about 250 thousand hectares in the Northern Sudan were affected to some degree by sodicity or salinity (Ali and Fadl, 1977). The largest areas are mostly found to the north of Khartoum along both banks of the main Nile. These areas are located in the arid zone with relatively cool winters which favor the growing of high value fruits and vegetables. Salt affected soils of the Sudan were often found in Khartoum province, along the main Nile. Gezira clay plain and the White Nile and Northern states.

In Sudan , there are three soil orders affected by salts, namely: Vertisols, Aridisols and Entisols. The flood plains and upper terraces are affected to various degrees by salinity and sodicity (Blockhius *et al*., 1964; Soil Survey Staff, 1979).

Sodium was found to be the dominant cation in the area extending between Gezira in central Sudan to Wadi Elkhowi in the North:

1. Managil and North Gezira: Sulphates alone or together with carbonates are the dominant anions in the top soil, whereas in the sub-soil sulphates are dominant.
2. Khartoum and Shendi: Chlorides and sulphates are dominant anions in the top soil.
3. Eldamer: This is similar to the second group except in the subsoil, chlorides are dominant.
4. Wadi Elkhowi: Sulphates are dominant in the top soil while in the subsoil, chlorides and sulphates are dominant.

Salt effected soils in the Sudan, occur in the desert and semi desert zones e.g. the high terrace of the River Nile and its tributaries, and in arid regions e.g. central clay plain-north Gezira (Hag Abdalla, 1986). Most of salt affected soil in the Sudan, have relatively low nutrient status, they contain 0.01-0.02% organic nitrogen. The phosphorous in these soils occurred mainly in inorganic form of calcium phosphate and they also have less trace elements (Mustafa, 1986).

2.9. Effect of salinity on faba bean:

Faba bean tolerated salinity up to 27.9ds/m NaCl. The length of shoots and roots and their water content as well as dry matter yield remained more or less unchange up to 9.3ds/m NaCl (Shaddad *et al*.,1990).

Faba bean genotypes varied in their tolerance to high levels of salinity (Cordovilla *et al.,*1995). Nitrogen fixation is more sensitive to salinity than plant growth (Cordovill,*et al*. 1994). Harbirsing and Prokash (1986) reported that germination percentage and early seedling growth varied greatly depending upon the variety and salinity level and they were markedly decreased at 8ds/m but Manchanda and Sharma (1991.*et al*) reported that EC of 8ds/m reduced the seed yield of faba bean and peas by 15% and 50%, respectively. Compared to the control salinity decreased root and shoot dry weights , root-shoot ratio, grain yield, nodule number, nodule leg hemoglobin content and K content and increased Na, Mg and Ca, in all parts of the plant (Sharma,1991;Hamada *et al*.,1994; Abd Elsamed, 1993).

The over salinity of the soil is one of the main factors that limits the spread of plants in their natural habitats. It is an ever increasing problem in arid and semi-arid regions (Shanon, 1986). Fisher and Turner (1978) estimate that arid and semi-arid lands represent around 40% of the earth's area. The property of salinity tolerance is not a simple attribute, but it is an outcome of various features that depend on different physiological interactions, which are difficult to determine. The morphological appearance presented by the plant in response to salinity, may not be enough to determine its effect, so it is important to recognize other physiological and biochemical factors, including toxic ions, osmotic potential, lack of elements and other physiological and chemical disorders, as well as the interactions between these various stresses (Munns, 1993, 2002; Neumann, 1997; Yao, 1998; Hasegewa *et al*., 2000).

From the results of the studies, which looked at the effect of salt stress on growth, one can notice a connection between the decrease in plant height and the increase in the concentration of sodium chloride (Beltagi *et al*., 2006; Mustard and Renault, 2006; Gama *et al*., 2007; Jamil *et al*., 2007; Houimli *et al*., 2008; Rui *et al*., 2009; Memon *et al*., 2010). Numerous studies showed the affection of leaf area negatively by using different concentrations of NaCl (Raul *et al*., 2003; Netondo *et al*., 2004; Mathur *et al*., 2006; Chen *et al*., 2007; Zhao *et al*., 2007; Yilmaz and Kina, 2008; Rui *et al*., 2009). The harmful influence of salinity on leaf number also increases with the increase in concentration, according to the studies held by Raul *et al*. (2003), Jamil *et al*. (2005), Gama *et al*. (2007), Ha *et al*. (2008). Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Bayuelo Jimenez *et al*., 2002; Jamil *et al*., 2005; Niaz *et al*., 2005; Saqib *et al*., 2006; Turan *et al*., 2007; Saffan, 2008; Rui *et al*., 2009; Taffouo *et al*., 2009; Memon *et al*., 2010). Changes in water relations of plants that are stressed by salinity can be seen in certain studies that confirm that, many plants undergo osmotic regulation when they are exposed to salt stress by increasing the negativity of the osmotic potential of the leaf sap (Rodriguez *et al*., 1997; Gama *et al*., 2007, 2009; Kaymakanova and Stoeva, 2008; Kaymakanova *et al*., 2008). Many studies confirm the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most important. The effect on photosynthesis can be gauged from the effect on the photosynthetic pigments. The results of specific studies (Sultana *et al*., 2000; Tort and Turkyilmaz, 2004; Misra et al., 2006; Murillo-Amador *et al*., 2007; Taffouo *et al*., 2010) clearly indicate that salinity reduces the content of photosynthetic pigments in treated plants. Protein content can also be affected negatively or positively, by salt stress. The results of certain studies (Sultana *et al*., 2000; Tort and Turkyilmaz, 2004; Beltagi *et al*., 2006; Chen *et al*., 2007; Kapoor and Srivastava, 2010) demonstrate a decrease, or increase, in protein content in plants treated with different salt concentrations.

CHAPTER THREE

MATERIALS AND METHODS

The research for this study was conducted during the winter season 2015/2016. It was comprised of two experiments. The first one in the tissue culture laboratory of the Department of Horticulture, College of Agricultural Sciences, Sudan University of Science and Technology, Shambat. The second one in the nursery of the Horticulture Division, Ministry of Agriculture, Khartoum. The objective was to investigate the effect of saline water on seed germination and vegetative growth of faba bean (*Vicia faba* L.).

Five concentrations of sodium chloride (NaCl) solution and distilled water as a control were used.

3.1- **The plant material and solutions:-**

1- Faba bean seeds cv.Agabat were washed, air dried and stored under room temperature until used. The solutions used comprised:

1- distilled water as a control.

2- 0.032 molar sodium chloride solution.

3- 0.063 molar sodium chloride solution.

4- 0.125 molar sodium chloride solution.

5- 0.250 molar sodium chloride solution.

6- 0.50 molar sodium chloride solution.

These solutions have the following characteristics:-

Solution concentration (M)	(ppm)	Water potential (-mPa)
0.00	0.00	0.00
0.032	1827	0.15
0.063	3653	0.30
0.125	7305	0.61
0.250	14610	1.22
0.500	29220	2.43

3.2. Experimentations:

3.2.1. The effect of salinity on germination:

The effect of the different concentrations of NaCl and the distilled water was studied in the first experiment. Fifty seeds were placed on a paper towel wetted by the corresponding solution in a petri dish and 5ml were added to each petri dish and kept at room temperature. The seeds were examined daily and washed by the respective solution to save guard against fungal infection. A seed was considered to have germinated when the radicle emerged through the seed coat and was visible to the naked eye. The germinated seeds were removed. The experiment was terminated when no germination would have occurred for three successive days.

3.2.2. The effect of sodium chloride (NaCl) solution concentration on vegetative growth:

In the second experiment the effect of 0.016, 0.032, 0.063, and 0.125 molar NaCl solutions and distilled water (as a control) were studied.

Five seeds were sown in each plastic bag filled by clay-sand mixture and water was added .The seedlings were thinned to one per bag after ten days. During this period the soil was wetted with water. After that the plants were irrigated with the appropriate solution. The plants were put outdoors until the cessation of the experiment.

3.3 Parameters measured and calculated:

The daily and final germination percentages were calculated as number of germinated seeds divided by the total number of seeds and then multiplied by 100.

The germination rate index (GRI) was calculated as the summation of the daily germination percentage divided by the total number of days of germination (Maguire, 1962). The corrected germination rate index (CGRI) was calculated as follows:

$$CGRI = \frac{GRI \ X \ 100}{\text{The final germination percentage}}$$

The number of days to reach 50% of the final germination (G T 50) was also determined.

The plant height and number of leaves per plant was determined. Also fresh and air dried plants weight was determined.

3.4. Experimental design and statistical analysis:

In the two experiments a completely randomized design (CRD) was used with each treatment replicated three times. Data collected were subjected to analysis of variance (ANOVA). Means were separated by the least significant difference (LSD). test at the 5% level.

CHAPTER FOUR

RESULTS

4.1. Effect of sodium chloride solution concentration on seed germination.

4.1.1. Effect on the daily germination percentage:

All treatments resulted in an increase in the cumulative germination percentage.(Fig.1).

4.1.2. Effect on the final germination percentage.

With the exception of the effect of the 0.250M Na Cl the final germination percentage was above 90% (Table 1). There was no significant difference among these treatments. However, there was a significant difference between these treatments and the 0.250 M Na Cl solution which was about 20% less than the rest of the germination percentages.

4.1.3. Effect on the seed germination rate.

The number of days to 50% (GT50) of the final germination percentage was lowest with the use of distilled water (Table 1). The difference between this and the other treatments was statistically significant (Table 1). On the other hand the 0.125M NaCl solution resulted in a significantly longer time than the other treatments. The difference was statistically significant. For the other treatments, the differences among them were not statistically significant. Both the germination rate index (CGRI) and the corrected germination rate index (CGRI) were highest for the distilled water treated seeds (Table 1). The deference between this and other treatments was statistically significant. In contrast the 0.125M Na

Cl solution resulted in the lowest values of the GRI and the CGRI. Here, too, the difference was statistically significant. Both the GRI and the CGRI were not affected significantly by the rest of the treatments (Table 1).

Table 1. Effect of sodium chloride (NaCl) solution concentration on final germination (%), number of days to 50% of the final germination percentage (GT50), germination rate index (GRI) and corrected germination rate index (CGRI) of faba bean.

NaCl concentration (M)	Final germination (%)	GT50 (days)	GRI % day^{-1}	CGRI (day^{-1})
0.00	93.33[a]	2.77[c]	52.67[a]	56.37[a]
0.032	93.33[a]	3.73[b]	43.13[b]	46.23[b]
0.063	90.67[a]	3.60[b]	44.00[b]	48.57[b]
0.125	90.67[a]	4.03[b]	40.53[b]	44.67[b]
0.250	70[b]	4.83[a]	22.78[c]	32.43[c]
LSD	11.43	0.55	7.03	5.91

Means in columns followed by the same letter are not significantly different at the 5% level using LSD.

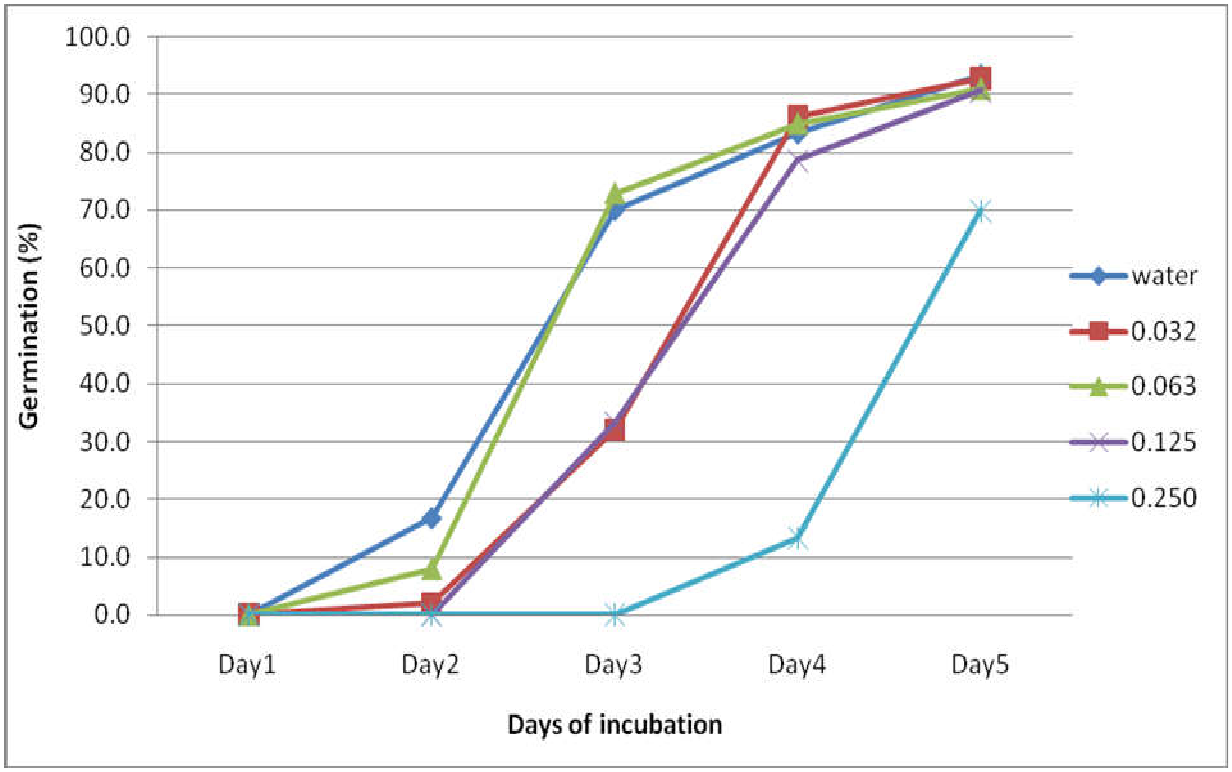

Fig.1. Effect of sodium Chloride (Na Cl) solution concentrations (M) on cumulative germination at different days of incubation.

4.2.1.Effect of sodium chloride on Plant height (cm):

The effect of sodium chloride (Na Cl) on the weekly height of bean plants is shown in (Table 2). At the end of the first week the heighest plants were those treated with 0.016 followed by 0.032 then 0.063 molar (M) Na Cl solution, whereas the least height was brought about by the distilled water and the 0.125 M Na Cl solution. At the end of the second week the plant height was almost the same for all solutions. At the end of the third week, the plants treated with the 0.125 M Na Cl solution were significantly shorter then the west of plants, which where almost similar in height. At the end of the fourth and the fifth weeks there was no change in plants height compared to the third week. However, the plants treated with 0.125 M Na Cl were dry and can easily be crushed (Table 2).

Table 2.Effect of sodium chloride on Plant height (cm) of faba bean plants treated with different sodium chloride (Na Cl) concentrations (M) at different times after emergence.

NaCl concentration(M)	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**
0.00	22.50	33.33	42.33	43.33	41.55
0.016	30.17	33.33	39.00	40.33	41.5
0.032	29.00	33.00	40.00	41.17	42.5
0.063	26.33	32.67	39.67	42.50	43.5
0.125	23.83	30.33	33.67	0.00	0.00

4.2.2: Effect of sodium chloride on number of leaves per plant:

At the end of the first week the number of leaves was about the same for the distilled water and the Na Cl solutions (Table 3). The number of leaves per plant increased substantially for the second week and the third week.

At the end of the fourth week, the number of leaves per plant was less compared to the third week, for all treatments with the exception of the distilled water treatment. At the end of the fifth week all treatments decreased the number of leaves per plant, compared to the fourth week with the plants treated the 0.125 M Na Cl solution were devoid of any leaves (Table 3).

Table 3. Effect of sodium chloride on Number of leaves per plant of faba bean plants treated with different sodium chloride (NaCl) concentrations (M) at different times after emergence.

Na Cl concentration (M)	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**
0.00	21.7	35.3	56.7	61.3	60.3
0.016	22.0	33.3	48.0	37.7	27.0
0.032	22.3	41.0	54.7	43.3	36.3
0.063	21.3	33.0	47.7	41.3	35.0
0.125	20.3	25.3	35.7	0.00	0.00

4.2.3: **Effect of sodium chloride on Fresh and dry weight per plant:-**

At the end of the fifth week, the fresh weight was almost the same for all treatments, except the 0.125 M Na Cl solution (Table 4 and Figure 2).Likewise was the dry weight per plant.

Table 4. Fresh and dry weight (g) of faba bean plant treated with different sodium chloride (Na Cl) concentrations.

Na Cl concentration (M)	Fresh weight (g)	Dry weight (g)
0.00	15.0[a]	9.83[a]
0.016	12.17[a]	9.00[a]
0.032	11.50[a]	9.33[a]
0.063	11.33[a]	7.66[a]
0.125	0.00[b]	0.00[b]
LSD	3.499	2.967

Means in columns followed by the same letter are not significantly different at the 5% level using LSD.

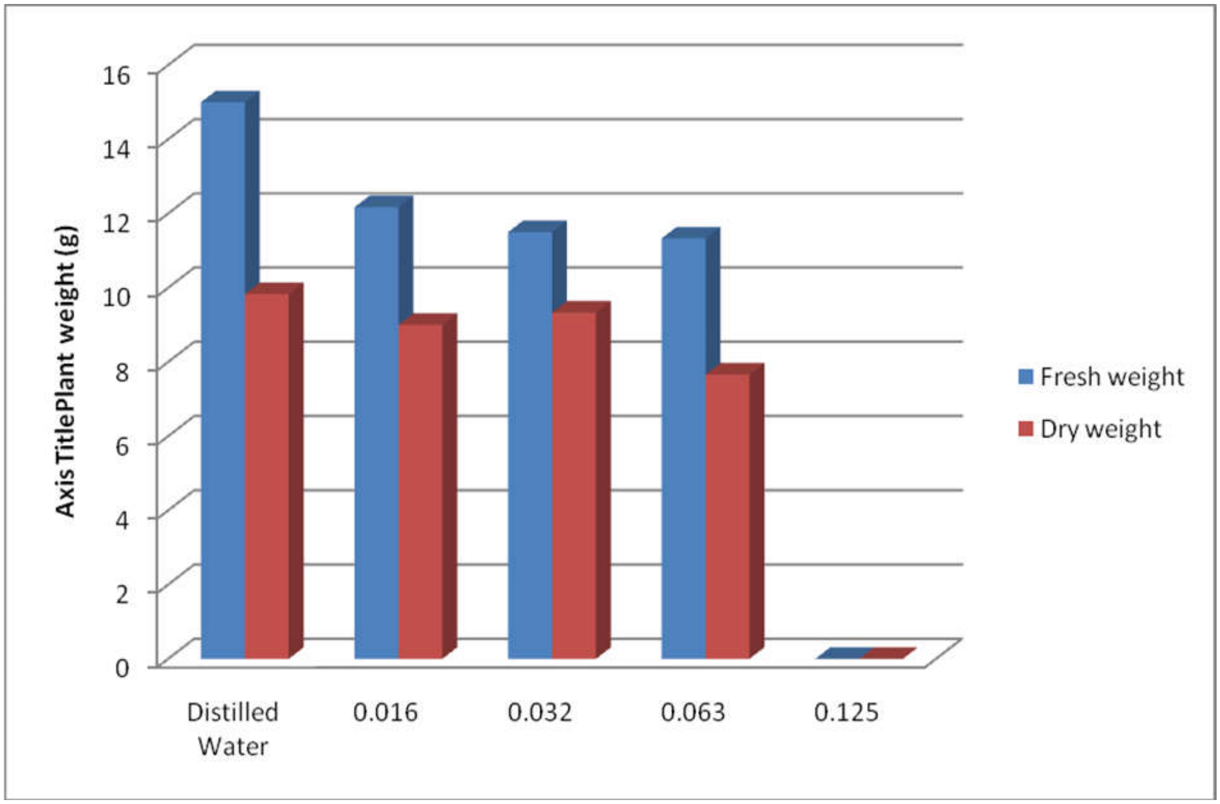

Fig.2. Effect of sodium chloride (Na Cl) concentrations (M) on fresh and dry weight (g) of faba bean plants.

CHAPTER FIVE
DISCUSSION

The germination inhibition of Faba bean (*Vicia faba* L.) with the highest salt concentration might by due changing in osmotic pressure or contribution to $Cl^{-}$and Na^{+} toxicity. Harbirsing and Prokash (1986) reported that germination percentage and early seedling growth varied greatly depending upon the variety and salinity level. Changes in water relations of plants that are stressed by salinity can be seen in certain studies that confirm that, many plants undergo osmotic regulation when they are exposed to salt stress by increasing the negativity of the osmotic potential of the leaf sap (Rodriguez et al., 1997; Gama et al., 2007, 2009; Kaymakanova and Stoeva, 2008; Kaymakanova *et al*., 2008).

The highest salt concentration tested, significantly decreased plant height at the early stages of plant growth compared with the other treatments tested and caused death of the plants at the later stages of plant growth indicating the relatively high sensitivity of elongation of shoots of broad bean to salinity. From the results of the studies, which looked at the effect of salt stress on growth, one can notice a connection between the decrease in plant height and the increase in the concentration of sodium chloride (Beltagi *et al*., 2006; Mustard and Renault, 2006; Gama *et al*., 2007; Jamil *et al.*, 2007; Houimli *et al*., 2008; Rui *et al*., 2009; Memon *et al*., 2010). Many studies confirm the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most important. The effect on photosynthesis can be gauged from the effect on the photosynthetic pigments. Sultana *et al*. (2000)Tort and Turkyilmaz(2004) Misra *et al*.(2006) Murillo-Amador *et al*. (2007) Taffouo *et al*.(2010) reported that salinity reduces the content of photosynthetic pigments in treated plants.

The diluted salt concentrations gave higher values of number of leaves relative to the control in the early stages of growth, but in the later stages of plant growth all salt concentrations tested significantly reduced the number of leaves compare to the control, the harmful influence of salinity on leaf number increases with the increase in concentration, according to the studies held by Raul *et al.* (2003), Jamil *et al*. (2005), Gama *et al*. (2007), Ha *et al*. (2008).

All salt concentrations tested resulted in a non-significant decreased in both parameters fresh and dry weights relative to the control. Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Bayuelo Jimenez et al., 2002; Jamil et al., 2005; Niaz et al., 2005; Saqib et al., 2006; Turan et al., 2007; Saffan, 2008; Rui et al., 2009; Taffouo et al., 2009; Memon et al., 2010).

Conclusion

It seems that the irrigation water with up to 0.125 molar sodium chloride may not affect the seed germination of faba bean seriously. However, the vegetative growth(the plant height and number of leaves) would be reduced at the time of flowering, fruit set and fruit development. More research is warranted to address the effect of saline irrigation water on this important leguminous crop especially in the Sudanese diet.

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