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The Germination Ecology and Composition of the Indigenous Seeds of Cyprus conglomeratus: A Sand Dune Binder

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The Germination Ecology and Composition of the Seeds of indigenous *Cyperus conglomeratus*: A sand dune binder

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ABSTRACT

Cyperus conglomeratus is among few species that tolerate the instability of sand dunes and its low nutrients. This species tolerates grazing animals and sand burial. It is considered as a good fodder for both domestic and wildlife animals. It has the potential to fix sand dunes. In addition, a preliminary observation indicated the presence of high contents of oil in the seeds. Despite the ecological importance of *C. conglomeratus,* little information is available about germination behaviour and seed composition. The present study assessed the dormancy, germination requirement, tolerance to salinity and drought during germination stage, the impact of dormancy regulation chemicals on innate dormancy and salinity-induced germination inhibition and seed composition of the glycophytic desert sedge *C. conglomeratus* seeds.

Fresh seeds of *C. conglomeratus* have little innate dormancy; 84% of the seeds germinated in distilled water. In addition, none of studied dormancy regulating substances improved seed germination. The effects of maternal habitat, time of seed maturation and seed storage, and their interaction were assessed on light and temperature requirements during seed incubation on final germination percentage and germination rate of *C. conglomeratus*. The results showed significant effects for maternal habitat, time of seed collection, seed storage and both light and temperature of seed incubation and their interaction on both germination percentage and rate. Seeds of *C. conglomeratus* produced on dry sand dunes (Al Wathbah, Al Khattem and Dubai) produced higher germination percentages whereas those produced in the two sites of Al-Ain (Manaseer and Industrial area) produced significantly lower germination. The lowest germination was recorded for seeds of Liwa population and this was attributed to the extremely water stress in that area which would lead to improper seed filling and high rate of seed abortion.

Both light and temperature of incubation significantly affected the final germination percentage and germination speed of *C. conglomeratus*. Germination in light was significantly greater than it in dark, especially at higher temperature. Germination at higher temperatures was significantly greater than that at lower temperatures. The light requirement for germination of *C. conglomerates* seeds at higher ensures that they will germinate successfully on or near the soil surface when other conditions are suitable for seedling emergence. Such result might explain the high seedling emergence observed in disturbed sand dunes, especially after effective rainfall at the end of the rainy season.

The effect of salinity, light and temperature of incubation and their interaction had significant effects on final germination percentage of *C. conglomeratus*. The germination was greatly reduced in 25 mM NaCl and completely inhibited in 50 mM NaCl. Salinity tolerance was greatest in darkness at higher temperatures. For all salinities, no seeds were recovered at the lower temperatures and recovery germination percentage was significantly greater at 30/40 °C than 25/35 °C. Fusicoccin, GA3 and kinetin, completely alleviated salinity induced dormancy in all salinity levels. Nitrate and thiourea completely alleviated the germination inhibition in lower salinities, but partially alleviated it in 100 mM NaCl.

The effect of Polyethylene osmotic pressure on the germination of *C. conglomeratus* seeds was significant. The final germination was 90% in -0.1 MPa then decreased to 67%, 57% and 44% in -0.2,-0.3 and -0.4 MPa, respectively, and completely inhibited in -0.5 MPa. This result indicates that the germination inhibition in saline solutions is more likely due to osmotic effect, rather than ion toxicity.

Final germination of fresh seeds of *C. conglomeratus* was significantly greater, compared to it for seeds stored for 15 months. This result indicates that storage either induced a secondary dormancy or resulted in viability loss of the seeds.

There was no compositional analysis data available for the seeds of this plant. Seeds collected from the plants in the sand dunes of Abu Dhabi, Dubai and Al-Ain were found to contain moisture – 2.51%, ash – 7.74%, fat – 29.5% and protein – 13.5%. The seeds of this plant are rich in oil and the fatty acid profile of the soxhlet extracted fat was determined by capillary gas chromatography and found to be: C10:0 – 0.10, C12:0 – 0.10, C14:0 – 0.16, C16:0 – 7.47, C16:1 – 0.12, C18:0 – 3.52, C18:1 – 73.8, C18:2 – 13.14, C18:3 – 0.56, C20:0 – 0.12, C20:1 – 0.27, C22:0 – 0.39, C22:1 – 0.17. Lipids were also extracted using supercritical fluid extraction (SFE) and SFE conditions of 50 °C and 400 bar gave maximum yield of 28.6%. The fatty acid profile of SFE oil was similar to that of the soxhlet extracted oil. The mineral composition (mg/kg) was found to be as: Na: 297, K: 3029, Ca: 896, Mg: 2303, Fe: 191, P: 3491, Cu: 9.57 and Zn: 53.3. Heavy metal contaminants (mg/kg) – Pb: 0.23, Cd: 0.14 were found to be within acceptable limits. The study indicates that the seeds are highly nutritious and the seed oil is rich in unsaturated fatty acids. The seeds could serve as a highly nutritious feed for animals and poultry.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1. Desert Conditions and Seed Germination

The Arabian Peninsula, including the United Arab Emirates (UAE), experiences some of the most extreme climatic conditions found on the Earth. It is characterized by low, erratic rainfall, high evaporation rates and amongst the highest temperatures on Earth (Boer, 1997; Zahran, 1997; Ghazanfar & Fisher, 1998). In addition, high rate of evaporation is increasing soil salinity. In certain areas, the water table is high and a high rate of evaporation causes an accumulation of salts in the soil. Over the centuries these extreme conditions have applied stringent evolutionary selection pressures resulting in a uniquely plants adapted to extreme environmental conditions (Peacock *et al.*, 2003). In addition, such harsh conditions have led to differential life strategies for desert plants in order to maximize their fitness (Kigel, 1995). Variation in seed dormancy in plants of such heterogeneous, unpredictable deserts can be an important factor for increasing their genetic diversity, making them able to respond to environmental changes (Lacerda et al. 2004). For example, time of germination determines the environment in which the plant will develop, and eventually the fitness of the plant (El-Keblawy 2003a). The moment of germination can determine when reproduction and fruit ripening will occur (Kalisz, 1986; Biere, 1991; Stratton, 1992; Galloway, 2001a; Luzuriaga et al., 2006). Environmental control of germination acts through the seed coat, the endosperm, and resource and hormone supply (Baskin & Baskin, 1998; El-Keblawy and Lovett-Doust, 1998).

Soil salinisation is one of the most challenging environmental and ecological problems that are facing humankind and is increasing, especially in arid and semi-arid regions because of low rainfall and high rates of evaporation (Tobe *et al.*, 1999). Salinity problem is affecting 20% of the world's cultivated land and nearly half of the area under

irrigation (Sosa *et al.*, 2005). This problem is more acute in arid and semi-arid regions (Sandro *et al.*, 2006). Grainger (1990) concluded that the amount of irrigated land that desertified annually as a result of salinity increase is equivalent to the amount of newly irrigated land. One of the most important issues in water management is the maximization of the profitability from water use in adopting native plants in desert greening and as forages, especially in countries facing freshwater shortage and increase in ground water depletion and salinization. However, germination data are not available for most of the indigenous desert plants.

Seed germination is the initial and most critical stage in the life cycle of plants, especially under saline and arid conditions of the deserts (Khan and Gulzar, 2003). The success of desert plants under warm and dry conditions of subtropical arid habitats is primarily dependent on optimal conditions for germination and recruitment (Gutterman, 1994, Khan and Ungar, 2001; Khan and Gulzar, 2003, El-Keblawy 2003a). Several environmental factors that occur during seed wetting, such as amount and extent of rain, temperature, light, soil salinity, and soil nutrients (e.g., nitrate), all play roles in the regulation of the germination level and speed of non-dormant seeds. Generally, germination should occur at times of optimum combination of day length, temperature and salinity. Despite extensive studies on interaction effects of salinity and temperature on germination, the interactions between salinity and light and between salinity, temperature and light have not well studied (e.g., De Villiers et al., 1994; Khan and Ungar, 1997b; Gul and Weber, 1999; Gulzar et al., 2001, El-Keblawy and Al Rawai 2005).

1.2. Maternal effects on Germination

The conditions under which seeds mature on the mother plant can determine subsequent dormancy and responses of germination to environmental conditions, and consequently the fate of the next generation (Roach and Wulff 1987; Baskin and Baskin 1998). It has been documented that seed dormancy and germination responses vary greatly depending on maternal habitat and time of seed development and maturation on mother plants. Several studies have demonstrated that seed germination varies between populations of different species including, *Thymelaea hirsuta* (El-Keblawy et al 1996), *Acacia nilotica* (Krishan and Toky 1996), *Bromus tectorum* (Beckstead et al 1996, Meyer and Allen 1999a, b), *Coleogyne ramosissima* (Lei 1997), *Abutilon theophrasti* (Zhang and Hamill 1997), *Sinapis arvensis*, *Spergula arvensis* (Andersson and Milberg 1998), *Lepidium* sisymbrioids (Allen 1998), *Arabidopsis thaliana* (Andalo *et al.* 1998), 34 temperate *Carex* spp. (Schutz 1999), *Chamaecrista rotundifolia* (Xu et al 2000), *Cirsium pitchen* (Hamze and Jolls 2000), *Chamaecyparis thyoides* (Jull and Blazich 2000), *Portulaca oleracea* (El-Keblawy and Al-Ansari 2000), *Prosopis juliflora* (Keblawy and Al-Rawai 2006). In addition, considerable variation in germination behaviour was shown between three populations of 70 annual weeds (Milberg *et al.* 1996) and 23 (Milberg and Andersson 1998). Similarly, several studies have documented that environmental conditions experienced by maternal plants during the growing season play a significant role in determining subsequent germination rate and responses in seeds of many species, such as *Artemisia tridentata* (Meyer and Monsen 1991), *Spergularia marina* (Ungar 1988), *Portulaca oleracea* (El-Keblawy and Al-Ansari 2000), *Eruca vesicaria* (Pita Villamil *et al.* 2002), *Campanula americana* (Galloway 2002).

Temperature during seed development was shown as an important factor affecting seed germination. This had been documented both under field and experimental conditions in several

species (Harrington and Thompson 1952, Wurzburger and Koller 1976, Nosova 1981, Probert et al. 1985b, Alexander and Wulff 1985, Hacker and Ratcliff 1989, Sharif-Zadeh and Murdoch, 2000, Qaderi and Cavers 2000, El-Keblawy and Al-Ansari 2000, Jensen and Eriksen, 2001, Allen and Meyer 2002, Keblawy and Al-Rawai 2006, Keblawy et al. 2009). In addition, moisture condition at which maternal plants are growing has been shown to affect seed germination in some species (Kermode *et al.* 1986, Meyer and Allen 1999b, Allen and Meyer 2002). In most of these studies, seeds produced by plants at higher temperatures, water stress and long days have higher germination percentage and/or rates (i.e. lower dormancy) than those produced at lower temperatures, short days and favourable moisture conditions.

1.3. Effects of Salinity and drought on Germination

There are two broad groups of plants according to salt tolerance: halophytes (salt tolerant) and glycophytes (salt intolerant). The responses of seeds of halophytes to salt differ from those of glycophytes. Halophytes can grow and complete their life cycle in saline environments, whereas glycophytes cannot (Ungar, 1991). Increasing salinity generally reduces germination in glycophytes and to a lesser degree in halophytes (Hayward and Bernstein, 1958; Waisel, 1972; Ungar, 1991, 1996; Khan and Ungar, 1997, Meloni et al., 2008). In addition, compared with glycophytes, seeds of halophytes germinate at higher salinities, and maintain viability even under extreme salinity or osmotic stress, recovering and germinating when the water potential of the medium increases (Ungar, 1995). However, seeds of some glycophytic plants are able to recover germination when exposed to salinity solutions (Tlig et al. 2008).

Many halophytes have the ability to maintain their seed viability after exposure to hyper-saline solutions and then initiate germination when salinity stress is reduced. These include *Atriplex patula* (Ungar, 1996), *Suaeda fruticosa* (Khan and Unger, 1997), *Arthrocnemum macrostachyum, Sarcocornia fruticosa* and *Salicornia ramoissim* (Pujol *et*

al., 2000) *Salicornia rubra* (Khan *et al.*, 2000). In other halophytes, recovery germination from higher salinities was very low (e.g., *Zygophyllum simplex*, Khan and Ungar, 1997; *Halogeton* glomeratus, Khan *et al.*, 2001a; *Sarcobatus vermiculatus* (Khan et al., 2001d), *Sporobolus ioclades*, Khan and Gulzar, 2003). The variation in recovery responses was attributed to differences in the temperature regime to which they are exposed (Khan and Ungar, 1997; Gulzar and Khan, 2001; Khan *et al.*, 2001a; Gul and Weber, 1999). However; the impact of saline solutions on the ability of seeds of glycophytes is not well studied (but see Tlig et al. 2008). Here, we assume that seed viability of glycophytes is greatly reduced after exposure to higher salinity.

Two processes mediate germination reduction in seeds experience higher levels of salinity: osmotic effects due to declining soil solute potential, creating a water stress for the plant, and ionic effects due to seed or seedling ion uptake and/or accumulation (Hayward and Bernstein, 1958; Waisel, 1972; Ungar, 1991). Salinity-induced declines in germination are usually due to only osmotic effects for halophytes, whereas glycophytes are more likely to exhibit additional ion toxicity (Hayward and Bernstein, 1958; Ungar and Hogan, 1970; Macke and Ungar, 1971; Cluff, Evans, and Young, 1983; Romo and Haferkamp, 1987; but see Dodd and Donovan 1999). Ionic effects may be distinguished from osmotic effects by comparing the effects of salt solutions and isotonic solutions of an inert osmotic medium such PEG that cannot penetrate into the cell wall. Inhibition of germination in PEG-treated seeds is attributed to osmotic effects, and any difference in germination of salt-treated relative to PEG-treated seeds is attributed to ionic effects. The general lack of ion toxicity for halophytes has been alternatively confirmed by almost complete recovery of germination potential after salt-treated seeds are returned to fresh water (Ungar, 1996; Egan, Ungar, and Meekins, 1997).

Despite most of the studies have examined salinity tolerance for halophytic plants of the arid lands, few studies tested the germination behavior of glycophytic desert plants. Such studies are important as big parts of the deserts are suffering from the salinity increase. In addition, some of the native plants could be useful in reseeding the degraded arid lands, especially those affected by higher salinity, or to replace exotic plants that are currently used as fodder for animals.

Drought is an environmental stress that adversely affects plant growth and crop. It is a period of below normal precipitation that limits plant productivity in a natural or agricultural system (Boyer, 1982). Plant responses to drought stress at the molecular, biochemical and physiological levels (Munns 2002; Verdoy *et al.*, 2004; Barnabas *et al.*, 2008). The response of seeds to drought could be an indicator of the tolerance of plants for the later stages of development. Therefore, there have been attempts at germinating seeds under variable stress conditions to identify the populations which adapt to dryness. Polyethylene glycol (PEG) 6000 solutions are frequently used for producing a range of water potentials, as they are relatively non-toxic to seeds. Seed treated with PEG 6000 inhibit germination due to osmotic effect (Emmerich & Hardegree, 1990).

1.4. Interactive effects of Light, Temperature and Salinity on Germination

Temperature plays a major role in determining the periodicity of seed germination and the distributions of species (Baskin and Baskin, 1988). The establishment of plants in arid regions is often limited by the temperature even though the conditions of humidity are favourable (Evans and Etherington, 1990; Jordan and Haferkamp, 1989; Oberbauer and Miller, 1982). Temperature changes may affect a number of processes controlling seed germinability, including membrane permeability and the activity of membrane-bound and cytosolic enzymes (Bewley and Black, 1994; Gul and Weber, 1999).

Light is another important regulatory environmental signals in seed germination of desert plants. Seeds are sensitive to light intensity, spectral composition of light and the duration of exposure to light (Baskin and Baskin 1998). Desert plants vary in their response to light during germination. Some have an obligate requirement for germination, while in others, presence of light enhances seed germination to varying degrees, and still others do not require light for germination. Baskin and Baskin (1995) demonstrated that of 41 halophytic species, light promoted germination in 20, darkness in 10 and 11 species germinated equally well in both light and dark. It is generally regarded that a light requirement prevents germination of seeds buried too deep for seedling to emerge because physiologically active light flux densities rarely penetrate more than a few millimeters into soil (Pons, 1992). In many species the light requirement for germination may vary with temperature. Buried seeds could detect their position in the soil by limiting their germination in dark and at lower temperatures. However, light requirement and high temperatures indicate seed location on or near the soil surface (Milberg *et al*., 2000). Hence, seeds requiring light will not germinate when they are buried under soil or leaf litter, but will germinate when exposed on the soil surface.

Light and salinity have interacted to determine germination in some halophytes. For example, the increases in NaCl concentration progressively inhibited seed germination in *Allenrolfea occidentalis*, and this inhibition was greater in the dark than in light (Gul and Weber, 1999). Similarly, germination was inhibited in four desert shrubs and a forb species with an increase in salinity, and this inhibition was more substantial in dark than in light (Khan and Ungar, 1997b). Also, both dark and high salinity also inhibited germination of *Limonium stocksii* (Zia and Khan, 2002). However, the effect of salinity did not depend on light conditions in the invasive *Prosopis juliflora* in the deserts of the UAE (El-Keblawy and Al Rawai 2005).

Establishment of species in salt deserts is related to germination response of seeds to salinity and temperature and early establishment usually determines if a population will survive to maturity (Huang et al., 2003; Song et al., 2005; Tobe et al., 2000). Temperature interacts with salinity to determine germination level and speeds of many species of the arid lands. Although higher salinity decreases germination, the detrimental effect of salinity is generally less severe at moderate temperatures in many species such as *Sporobolus ioclados* (Khan and Gulzar, 2003), *Urochondra setulosa* (Gulzar et al., 2001), *Ducrosia anethifolia* (Al-Yemeni and Basahy, 1999) *Zygophyllum simplex* (Khan and Ungar, 1997a), *Crambe abyssinica* (Fowler, 1991), *Urochondra setulosa* (Gulzar *et al.*, 2001), *Salsola imbricata* (El-Keblawy *et al.*, 2007). However, the detrimental effect of salinity was severe at higher temperatures in some species including *Atriplex cordobensis* (Aiazzi et al., 2002), *Sarcobatus vermiculatus* (Khan et al., 2002a), *Polygonum aviculare* (Khan and Ungar, 1998) *Sagittaria latifolia* (Delesalle and Blum, 1994), *Atriplex semibaccata* (De Villiers et al., 1994) and at lower temperatures for other species including *Halopyrum mucronatum* (Khan and Ungar, 2001a); *Aeluropus lagopoides* (Gulzar and Khan, 2001), *Salicornia rubra* (Khan et al., 2000), *Allenrolfea* (Gul and Weber, 1999), *Zygophyllum qatarense* (Ismail, 1990). In addition, salinity tolerance did not depend on temperature in some other species such as *Arthrocnemum indicum* (Khan and Gul, 1998). The salinity-temperature interaction may have significant ecological implications in terms of time of germination under field condition (Ungar, 1995).

1.5. Impacts of Dormancy Regulating Chemicals

Salinity prevents germination of seeds either by reducing water availability or interfering with some aspects of metabolism, such as altering the balance of growth regulators (Khan and Ungar 2001a). Plant growth regulators play important roles in seed germination.

Salinity stress is usually associated with the inhibitory effect of abscisic acid (ABA) and the decline in cytokinin concentration (Esashi et al. 1979). ABA is involved in induction and maintenance of seed dormancy in many plants; whereas, the gibberellins have actions that appear to be antagonistic to ABA (reviewed by Bewley and Black, 1994). Several studies have shown the ability of dormancy regulating chemicals to alleviate salinity induced dormancy in many species. Nitrogenous compounds, such as thiourea and nitrate, are known to counteract the inhibitory effect of ABA and the decline in cytokine concentration associated with salinity stress. Consequently, such chemicals alleviate salinity induced inhibition of germination (Esashi et al. 1979). Thiourea was effective in alleviating the inhibition of germination by salt or high temperature stress (Esashi *et al.*, 1979; Bewley and Black, 1994; Khan and Ungar 2001a, b, c). In addition, nitrate is known to stimulate the seed germination of many species (Stokes, 1965; Mayer and Poljakoff-Mayber, 1975) and it received considerable attention as a possible regulator of seed germination in the soil (Egely, 1995). A mixture of nitrate and ethephon stimulated germination of *Chenopodium album* seeds (Karssen, 1976; Carmona and Murdoch, 1995). In addition, the application of GA3 (Khan and Ungar 1997, 1998), kinetin (Khan and Ungar 1997), fusicoccin (Ismail 1990; Gul and Weber 1998), and ethylene (Kepczynski and Karssen 1985; Kepcyznski 1986; Ismail 1990) have been shown to alleviate salinity-enforced dormancy. Whereas several studies assessed the impacts of dormancy regulating chemicals on salinity induced dormancy of halophytes few studies did that for glycophytes. It is important to assess the differential effects of various germination regulating chemicals to better understand the germination inhibition mechanisms under saline conditions for glycophytic plants.

1.6. Impact of Seed Storage on Germination

Many species produce seeds that do not germinate shortly after dispersal and require a period of species-specific after-ripening through dry storage (Bewley and Black, 1982; Simpson, 1990; Baskin and Baskin, 1998). The parameters that determine seed after-ripening are moisture and oil contents, seed-covering structures, and temperature (Manz *et al.*, 2005 and references therein). As a general rule, at the time of natural dispersal from mother plants innate dormancy will be expressed and germination requirement will be highly specific. In this case, environmental factors during storage will program the seeds to germinate under certain environmental cues so that germination occurs at the appropriate time (Probert, 1992). Non-dormant seeds germinate upon water uptake if they are exposed to favorable environmental conditions.

1.7. Composition of *C. conglomeratus* seeds

The composition of C. *conglomeratus* plant [Ash-10.9%, Silica- 1.9%, Fat-2.2%, Protein-5.8%, Lignin-4.9%, ADF-42.4, NDF-70.6% (results expressed on dry weight basis)] was reported in a handbook published by the College of Food and agriculture of the UAE University (Alhadramy et al. 2000). However, there is no available information on the composition of seeds, its oil content as well as the fatty acid composition of oil.

1.7.1 Extraction of Oil using Soxhlet Technique

Solvent extraction of solid samples, which is commonly known as solid-liquid extraction, is one of the oldest ways of solid sample pretreatment. Among the techniques used for extracting oil, Soxhlet extraction technique is most commonly used for a long time. This assertion is supported by the fact that Soxhlet has been a standard technique during more than one century and, at present, it is the main reference to which the performance of

other leaching methods is compared. In conventional Soxhlet, originally used for the determination of fat in sample, the sample is placed in a thimble-holder, and during operation gradually filled with condensated fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solute of the thimble-holder and unloads it back into the distillation flask, carrying the extracted oil along with the solvent into the bulk liquid. This operation is repeated until complete extraction of oil is achieved. This performance makes Soxhlet a hybrid continuous-discontinuous technique. In as much as the solvent acts stepwise, the assembly can be considered as a batch system; however, since the solvent is recirculated through the sample, the system also bears a continuous character (Soxhlet and Dinglers, 1879).

The most outstanding advantages of conventional Soxhlet are as follows: the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium. The temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent. No filtration is required after the leaching step. Sample throughput can be increased by simultaneous extraction in parallel, since the basic equipment is inexpensive (International Organization for Standardization,1988).

It is a very simple method and has the possibility to extract more sample mass than most of the latest methods and is nonmatrix dependent. This technique has not been used before with the plant of *Cyperus conglomeratus* and it will be very interesting to investigate the quantity of oil included in the composition of this plant (Soxhlet and Dinglers, 1879).

1.7.2 Extraction of Oil using Supercritical fluid extraction (SFE) Technique

Oils that are extracted by using environmental hazardous organic solvents such as n-hexane or petroleum ether might contain residual solvent because of the incomplete solvent removal. Therefore, there is a need for a more environmentally friendly and safer solvent. Supercritical carbon dioxide fluid extraction seems to be a good solution since it is non-toxic, non-explosive, environmental friendly, inexpensive, time saving and its solvating power could be adjusted by simple changing in the temperature and pressure (Vaquero, Beltran, & Sanz, 2006).

Above its critical point (304.15Kand 7.38MPa), where the distinction between a liquid and a gas disappears, the density of CO_2 can be varied by almost an order of magnitude with relatively small changes in temperature or pressure, so its solvating power can be regulated and controlled . This property allows easy downstream separation. In addition CO_2 immediately evaporates when brought to atmospheric conditions, producing oil that's free from chemical and thermal degradation compounds and from solvent residue. CO_2 extracts are commonly known as safe (GRAS) to be used in food products (Gerard & May, 2002). Therefore, SFE may serve as a very promising and clean technology in food and pharmaceutical processing (King, 2000).

Furthermore, it offers the oil to be extracted at low temperatures and complete removal of solvent at the final stage of the extractions (ozkal, Salgın, & Yener, 2005). Extraction of fats and oils from various natural sources (vegetable, nuts, seeds, spices, meat products, marine products, skin, etc.) by using SFE technique has been broadly reported and reviewed in the scientific literatures (Marrone *et al.* 1998).

However SFE has not been used on *C.conglomeratus* before, therefore it will be very interesting to investigate the results of this technique on the yield and quality of extracted oil.

1.7.3 The Compositional Analysis

The compositional analysis (moisture, ash, oil content, protein, carbohydrates) of *Cyprus conglomeratus* seeds is carried out using official standard methods published by the Association of Official Analytical Chemists (AOAC)) Crude fiber, ADF and NDF are determined following American Oil Chemists Society (AOCS) approved procedures using ANKOM automated fiber analyzer (ANKOM, 1000A).

Moisture: The need to control moisture is set by the fact that the more water there is, the more liable it is to microbial spoilage. Water is not released from different states of its existence with equal ease and therefore different methods are used for its determination with different foods.

Moisture is determined by weighing an amount of the sample and place it in an oven maintained at 105 $\pm$ 2 ^{o}C and the change in the sample weight is monitored. When the sample weight is constant, the loss in weight of sample is calculated as percent of moisture in the sample.

Ash: Inorganic elements make up only 4% of animal body tissue, but they are essential as structural elements and in many vital processes. The main roles of these elements can be described as functional and structural. From the functional standpoint, they play a catalyzing role in enzymatic systems by binding their ions to substrates.The proportions and quantities in animal body tissue are different so inorganic elements are classified as macro-elements (elements the body needs in large quantities) and micro-elements (elements it requires in smaller amounts). Twenty-five of the chemical elements in the periodic table can be classified as essential. However, in practical terms, the macro-

elements considered essential are calcium, phosphorus, magnesium, potassium, sodium. chlorine and sulfur, while the essential microelements are iron, iodine, selenium, cobalt, manganese, zinc and copper (Watanabe, Kiron, & Satoh, 1997).

The ash values of foods represent their mineral/metal content. It is important that feed ingredients as well as finished feeds have balanced mineral composition and be free from toxic heavy metals such as lead, cadmium, arsenic and mercury. Total Ash is the inorganic matter, left as a residue or after the food is subjected to incineration at 500-550°C. This matter is composed of mineral salts in the form of oxides, halides, carbonates, phosphates, sulphates etc. The organic matter of a known quantity of the sample in a pre-weighed silica crucible is destroyed by incinerating at 450°C and the residue is calculated as per cent total ash.

Lipids: are one of the large groups of organic compounds, which are of great importance in our food as they provide heat and energy. Fats are water insoluble substances, which consist of the glycerol esters of fatty acids called glycerides. They occur in foods in free as well as bound form. They are also present as simple lipids and compound lipids such as phospholipids and glycolipids. They are widely distributed and almost every natural food has considerable quantities of fat. Fruits and vegetables are not ordinarily thought of as a source of fats, but the natural foods that contribute the largest amounts are the animal products (meats and fowl), milk and milk products and eggs. Fat can be estimated by Continuous extraction method using Soxhlet type of apparatus.The natural fats and oil are mixtures of glycerides of fatty acids (Maitland, 1998).

Proteins: (also known as polypeptides) are organic compounds made of amino acids arranged in a linear chain and folded into a globular form. The amino acids in a polymer chain are joined together by the peptide bonds between the carboxyl and amino groups of adjacent amino acid residues (Ridley, 2006).

Carbohydrates: are simple organic compounds that are aldehydes or ketones with many hydroxyl groups added, usually one on each carbon atom that is not part of the aldehyde or ketone functional group. The basic carbohydrate units are called monosaccharides; examples are glucose, galactose, and fructose.Total carbohydrates are determined by the difference as follows: [100 – (% moisture + % ash + % protein + % fat)] for foods. In the case of ruminant feeds crude fiber is not included in the calculation as most cellulose is digestible by the animals (Matthews et al, 1999).

Calorific value: The calorific value (K. cals. /100g): Calorific value is calculated by multiplying protein and carbohydrates with a factor 4 and fat with a factor 9 and adding the values (Nazaroff and Harley ,2007).

Crude fiber, ADF and NDF: Crude fiber is determined by laboratory analysis and is mainly composed of lignin, which is found in the tissues of plants and cellulose mainly a plant's skeleton. Whereas the ADF assay measures a portion of the cell wall, namely lignin and cellulose. In recent years, dairy producers have been encouraged to also have the neutral detergent fiber (NDF) content of forages determined. The NDF assay measures total plant cell wall material, and contains mainly hemicellulose, cellulose and lignin. Hemicellulose and cellulose are slowly digested by rumen microbes, whereas lignin is indigestible. Lignin is also cross-linked with other cell wall constituents, rendering them indigestible as well (Linn and Martin, 1991).

1.8. Study Species and Objectives of the Study

Cyperus conglomeratus is among few species that tolerate the instability of sand dunes and low nutrient content of its soils (El-Keblawy et al. 2009; Ksiksi et al. 2007). This species is the most dominant plant on overgrazed sand dunes of the UAE, indicating that it tolerates grazing animals and sand burial. This could be attributed to the presence of the

perennating buds on underground rhizomes under the soil surface. The individuals of this species make almost monospecific culture on overgrazed sand dunes (Ksiksi *et al.* 2007). El-Keblawy et al. (2009) indicated that camels prefer the new growth of *C. conglomeratus*. This species is considered as a good fodder for both domestic and wildlife animals species (El-Keblawy 2003b). In addition, *C. conglomeratus* can benefit from any disturbance and has the capacity to colonize sandy habitats before any other species following rain events and overgrazing (Ferguson et al., 1998). Moreover, the plants of this species have shallow roots with a sandy sheath enabling them to fix sand particles and absorb water precipitated through fog and dew falls. The use of *C. conglomeratus* in sand dune fixation could hinder the movement of the dunes in the plantations of the surrounding areas and, hence, in combating desertification. The plants of this species are evergreen, even during summer and early autumn, when the temperatures are high and rainfall is a rare event in the UAE deserts.

Morphological description of C.conglomeratus

Cyperus belongs to Cyperaceae, subfamily Cyperoideae, tribus Cypereae. Cyperaceae are one of the largest families of vascular plants, with about 4000 to 5000 species in 70 to 105 genera (Goetghebur, 1987; Kukkonen, 2001). Rhizome is short or long-creeping. Roots are tomentose or nearly glabrous. Stems are 2-90 cm, terete or subtrigonous. Leaves are basal, from shorter to longer than stem, from flat to nearly unifacial, basal sheaths are soft, or stiff and hard(Kukkonen 1994) (Figure 1).

Figure 1: *Cyperus conglomeratus* growing in the natural habitat

Despite the ecological and economic importance of *C. conglomeratus,* little information about germination behaviour is available in the literatures. Interestingly, our observation indicated that seeds of *C. conglomeratus* are very rich with fats. However, no study assessed seed composition of this species. The aims of the present study were, therefore, to (1) asses the maternal effect on temperature and light requirement during germination, (2) the effects of salinity, temperature and light of incubation and their interactions on salinity tolerance and recovery germination, (3) the impacts of dormancy regulation chemicals on innate dormancy and salinity-induced germination inhibition, (4) effects of seed storage on germination requirements of the glycophytic desert sedge *C. conglomeratus* growing in the UAE deserts, (5) assess the composition of seeds, its oil content as well as fatty acid composition of oil to evaluate its suitability for food/feed applications, (6) establish supercritical fluid extraction (SFE) conditions to obtain optimum yield and quality of oil, (7) asses of the quality of oil obtained by SFE and

solvent extraction techniques and (8) assess the compositional variation of the seeds collected from different areas within the Emirates

CHAPTER 2

MATERIALS & METHODS

2. MATERIALS & METHODS

2.1. Study Area

The United Arab Emirates is situated in Southwest Asia, bordering the Gulf of Oman and the Arabian Gulf, between Oman and Saudi Arabia; it is in a strategic location along southern approaches to the Strait of Hormuz. The UAE lies between 22°50′ and 26° north latitude and between 51° and 56°25′ east longitude. It shares a 530-kilometer border with Saudi Arabia on the west, south, and southeast, and a 450-kilometer border with Oman on the southeast and northeast.

In the present study, *Cyprus conglomeratus* seeds were collected from two sites in Abu Dhabi (24° 28′ 0″ N, 54° 22′ 0″ E), three sites in Al-Ain (24° 12′ 27″N, 55° 44′ 41″E) and one site in both Liwa (23° 8′ 0″ N, 53° 46′ 0″ E) and Dubai (25° 16′ 10.92″ N, 55° 18′ 34.2″ E). All the sites are sand dunes; however, those of Abu Dhabi (Al Wathbah, Al Khattem), Dubai and Liwa are considered to be dry dunes because of the high infiltration rate of the coarse sands. However, the two sites of the Al-Ain (Manaseer and Industrial area) are receiving extra water: the Manaseer site is irrigated for ornamental plants and the industrial site is nearby a site in which sewage treated water is dumped (Figure 2).

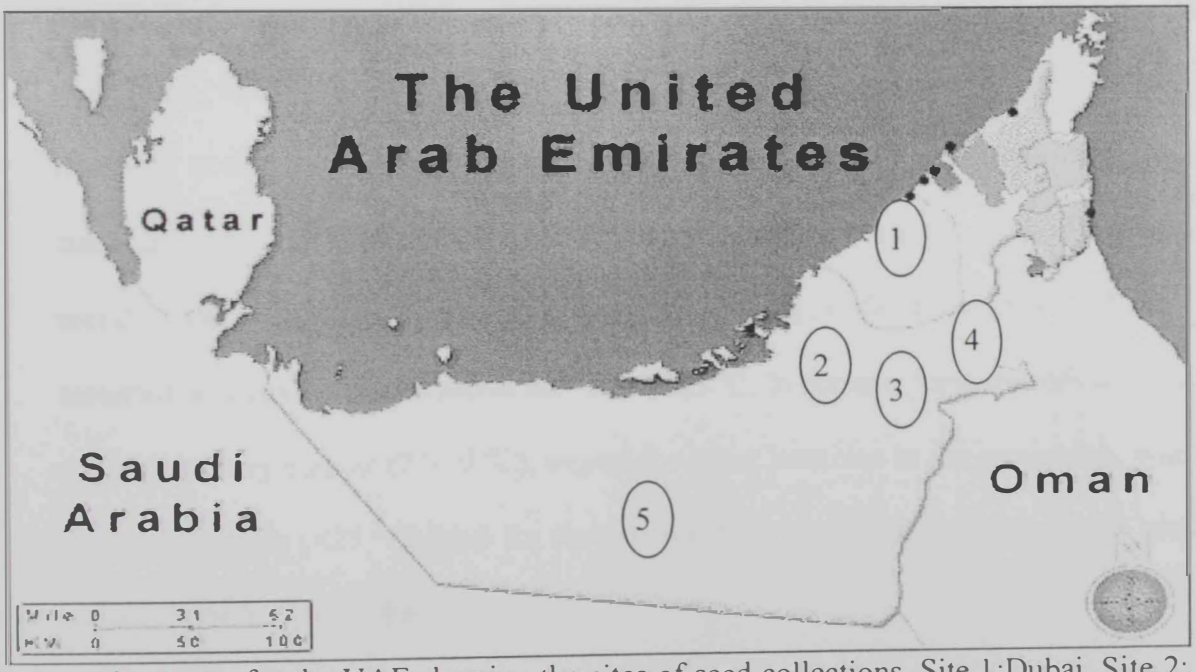


Figure 2: A map for the UAE showing the sites of seed collections. Site 1:Dubai, Site 2: Al Wathbha , Site 3: Al khettem, Site 4: Al Ain ,Site 5: Liwa

2.2. Climate of the UAE

The UAE is a subtropical hot desert country with high temperature and low rainfall. Its arid climate is characterized by two distinct seasons: a long dry summer season (April-November) with very high temperatures and a short winter season (December-March) mild to warm temperatures and a slight rainfall (Al-Asam, 1992; Embabi et al., 1993). The mean annual rainfall over the whole country is about 119 mm. This average can be highly variable between one year and another. The lowest annual rainfall is annually recorded in the western coast and inland desert: 97.7-105 mm/year and 90.7-128 mm/year respectively. The highest amount of rainfall is that of mountainous regions (mean annual = 125.6-172 mm) (Zahran, 1998, UAE Ministry of Agriculture & Fisheries, 1993).

Temperatures change throughout the seasons, and the coastal zones are much more buffered, whereas high differences are found in the southern area. In winter (Dec, Jan, Feb) coastal areas are slightly warmer (>20 °C) than terrestrial areas, and the eastern mountains (18-20 °C). In spring (Mar, Apr, May) the mountain and Gulf coast are still relatively cool (>27 °C), but temperatures in the southern terrestrial areas and the east coast are relatively high (27-28 °C or higher). These are more obvious in the summer months (Jun, Jul, and Aug), when temperature of the central desert exceeds 35 °C, and the mountain and coastal temperatures stay below 33 °C. In autumn, temperatures all over the country are very similar (27-29 °C), expect for some locations in the mountains, where it is slightly cooler (<27 °C), and for the north-eastern coastal areas, where it is slightly warmer (>29 °C) (Boer, 1997).

The humidity throughout all seasons is higher closer to the Arabian Gulf and to the gulf of Oman, and lowers in the south, south-west, and in Al-Ain region. Mean annual relative

humidity is over 60% for Abu Dhabi, with winter months generally over 70%. Foggy days, i.e. with rising sand, are recorded in all months. In summer there is a high incidence of suspended dust throughout the country brought by the prevailing wind from the head of the Arabian Gulf (Western, 1989). The average annual number of sunshine hours over the UAE is 10 hrs/day with a mean maximum of 11.5 hrs/day in May and a mean minimum of 8.4 hrs/day in December (Zahran, 1997).

In the present study, seeds of *Cyprus conglomeratus* were collected in May 2008. Seed maturation is usually take place during April and May. The climate of the nearest meteorological stations to the study regions, where seeds collected for the present study, during the year of seed maturation and collection (2008) are outlined below.

2.2.1 Abu Dhabi regions

Abu Dhabi has a hot arid climate. Sunny blue skies can be expected throughout the year. The months of April through September are generally hot and humid with maximum temperatures averaging above 40 °C. The weather is cooler from November to March. This period also sees dense fog on some days.

Abu Dhabi received only about 60 mm rainfall during 2008; most of it was in January (52 mm) (Table 1). The maximum temperatures during seed development in Abu Dhabi were 34.7 and 40.7 °C during April and May, respectively (Table 2). The maximum relative humidities in Abu Dhabi during April and May were 75 and 65%, respectively (Table 3).

2.2.2 Al Ain regions

Al Ain is located in the Eastern region of Abu Dhabi Emirate just south of Dubai and east of Abu Dhabi. More rainfall and lower temperatures occur in the northeast than in the

southern and western regions. The monthly average rainfall around Al Ain was (100–120 mm) for the period 1970 to 1992.

Al Ain received only about 37 mm rainfall during 2008; most of it was in January (23.1 mm) (Table 1). The maximum temperatures during seed development, which occurs in April and May were slightly higher (38 and 42.7 °C during April and May, respectively) in Al-Ain, compared to that of Abu Dhabi (34.7 and 40.7 in April and May, respectively) (Table 2). The maximum relative humidities during the April and May were drier in Al-Ain (48 and 68%, respectively), compared to it in Abu Dhabi (Table 3).

2.2.3 Liwa region

Liwa Oasis is about 100 km south of the Arabian Gulf coast and 150 km SW of the city of Abu Dhabi, on the northern edge of Rub al Khali desert. It stretches about 100 km east-west, along an arch curved to the north. The nearest meterological station for this region is Madinat zayed. During 2008, Liwa is considered to be the driest places in the country. Madinat zayed received only about 8 mm rainfall during 2008; all pricipaitated in January (Table 1). The maximum temperatures during April and May 2008 were 32.6 and 41.9 °C (Table 2). The maximum relative humidities during the April and May 2008 were 75 and 68%, respectively (Table 3).

2.2.4 Dubai region

Dubai is the second largest Emirate in the seven-member state and has a coastline that stretches almost 72 km along the south eastern shore of the Arabian Gulf. (Howari, 2004; Al-Darwish et al., 2005a,b).Dubai has a hot arid climate. Rainfall is generally light, with a mean of about 150 mm per year; precipitation is usually centered around the months of January, February and March. The long term mean humidity in Dubai is approximately 60% and is higher during the cooler winter months. During 2008, Dubai received the

greatest rainfall (135.8 mm), compared to the other sites. The maximum temperatures during April and May 2008 were 34.1 and 39.5 °C (Table 2). The maximum relative humidities during the April and May 2008 were 70 and 54%, respectively (Table 3).

Table 1: Total monthly rainfall (mm) for Abu Dhabi, Al Ain, Dubai and Madinat Zayed (nearest station for Liwa) regions during 2008.

Month	Abu Dhabi	Al Ain	Dubai	Madinat Zayed (Liwa)
January	52.0	23.1	108.8	8.0
February	0.0	0.0	0.2	0.0
March	0.0	0.0	0.0	0.0
April	0.0	0.0	0.0	0.0
May	0.0	0.0	Trace	0.0
June	0.0	0.0	0.0	0.0
July	0.0	5.1	0.0	0.0
August	0.0	2.4	0.0	0.0
September	0.0	6.2	0.0	0.0
October	0.0	0.0	0.0	0.0
November	Trace	0.0	1.6	0.0
December	7.8	0.0	25.2	0.0
Total	59.8	36.8	135.8	8

Table 2: Monthly average maximum and minimum temperature (°C) for the studied regions during 2008

Month	Abu Dhabi		Al Ain		Dubai		Madinat Zayed (Liwa)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
nuary	22.4	13.3	23.3	12.0	22.2	14.8	21.9	11.7
ruary	24.6	12.8	26.8	12.6	24.6	15.3	24.3	11.4
rch	31.0	16.3	33.9	16.4	30.7	18.5	32.5	15.0
ril	34.7	20.4	38.0	20.7	34.1	22.7	32.6	21.0
y	40.7	25.6	42.7	26.7	39.5	28.1	41.9	25.3
ne	39.2	26.5	43.5	27.0	38.6	28.4	42.6	26.3
y	42.7	29.4	45.7	30.5	42.2	31.8	45.2	27.9
gust	43.5	30.8	44.4	30.8	41.9	32.0	44.6	29.6
tember	40.1	28.0	41.6	26.9	38.9	29.6	41.4	25.9
tober	37.0	23.9	38.2	24.0	36.5	26.0	37.6	22.6
vember	30.6	19.5	31.7	18.9	30.9	22.0	30.5	17.7
cember	24.2	13.2	25.5	11.8	24.6	15.8	24.1	11.0

Table 3: Monthly absolute maximum and minimum relative humidity (%) for the studied regions during 2008

Month	Abu Dhabi		Al Ain		Dubai		Madinat Zayed (Liwa)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
January	85	43	88	49	75	41	92	47
February	81	33	79	30	72	29	90	30
March	81	26	72	20	76	25	81	13
April	75	22	67	17	70	24	75	28
May	65	14	48	17	54	17	68	11
June	74	25	68	20	68	27	65	10
July	76	20	66	19	66	21	84	10
August	69	21	64	26	65	24	79	17
September	76	23	77	23	70	28	83	16
October	82	22	70	24	73	24	94	18
November	79	33	85	37	69	31	93	31
December	86	46	93	43	77	42	94	37

2.3. Effect of site of origin

In order to assess the effect of the prevailing environment conditions during seed development and maturation at different populations on seed dormancy and germination behaviour, matured seeds of *Cyperus conglomeratus* were collected from different regions of the UAE during May 2008. The eastern region of the country was represented by 3 sites in Al-Ain region (Zakhir, Manaseer and Al Ain Industrial site), the western region was represented by two sites in Abu Dhabi (Al Khettem and Al Wathbah) and one site in Liwa and the northern region of the country was represented by one site in Dubai. Seeds were randomly collected from the whole population to represent the genetic diversity of the population. Seeds were separated from litters by using a series of sieves and dry stored in brown paper bags in room temperature until their use in the germination during September 2008.

Germination was carried out for all seeds collected from the different populations (maternal habitats). Germination was conducted using 90 mm plastic Petri dishes containing one disk of Whatman No.1 filter paper moistened with 10-12 ml distilled water. Seeds of each site were germinated in four incubators: three with alternating temperatures set at of 20/30, 25/35, 30/40 °C (12 h dark/12 h light) and the fourth at constant temperature of 30 °C. For each temperature, four replicates dishes, each with 25 seeds were wrapped in aluminum foil (continuous dark treatment), while other four were exposed to 12 h dark/12 h light (hereafter refereed as light treatment). The light source was from two 40 W daylight cool fluorescent tube and two 100 W incandescent lamps. In light treatment, germinated seedlings were counted and removed every second day for 20 days following seeds sowing. In the continuous dark treatment, dishes were wrapped in aluminum foil and opened after 20 days (at the end of the experiment). Radical emergence was the criterion for germination.

2.4. Effect of storage

In order to assess the impact of storage on light and temperature requirements during germination, seeds of *C. conglomeratus* were collected from Al Manaseer during May 2007 and germinated immediately after collection (within 2 – 10 days, hereafter it will be referred to as fresh seeds) and after 15 months of dry storage in brown paper bags at room temperature (hereafter it will be referred to as stored seeds). Germination was conducted in both light and dark at four temperatures adjusted as described above. In light treatments, germinated seedlings were counted and removed every second day for 20 days following seeds sowing. In the continuous dark treatment, dishes were wrapped in aluminum foil and opened after 20 days (at the end of the experiment). Radical emergence was the criterion for germination.

2.5. Interaction Effects of Salinity, Temperature and Light

Seeds of *C. conglomeratus* collected in May 2008 and stored for 4 months were used in this experiment. To determine the effects of salinity temperature and light and their interactions on final germination and germination rate, seeds were germinated in five salinities (0, 25, 50, 75, 100 mM NaCl) and incubated in five incubators adjusted at 10/20, 15/25, 20/30, 25/35 and 30/40 ℃ in both continuous darkness and in 12 h dark/12 h light. The salinity levels were selected based on a preliminary experiment tested the salinity tolerance of this species. The germination was conducted in 9-cm Petri-dishes containing one disk of Whatman No. 1 filter paper with 10 ml of test solution. Each dish was wrapped with Para film as an added precaution against loss of water by evaporation. Radical emergence was the criterion for germination. Germinated seedlings were counted and removed every second day for 20 days following seeds sowing.

After 20 days in the different salinity treatments, ungerminated seeds were transferred to distilled water in order to test their ability to retain viability under saline conditions. Germinated seedlings were counted and removed every alternative day for 10 days. The germination recovery index was calculated using the following formula (Khan *et al.*, 2000):

$$\text{Recovery percentage} = (a-b)/(c-b)*100$$

where "a" is the total number of seeds germinated after being transferred to distilled water, "b" is the total number of seeds germinated in saline solution, and "c" is the total number of seeds.

2.6. Effects of Dormancy Regulating Chemicals on Innate and Salinity Induced Dormancy

The effects of six different dormancy regulating chemicals on the innate dormancy as well as salinity induced dormancy of *C. conglomeratus* seeds was assessed by using six dormancy regulating chemicals and series of NaCl concentrations (0, 25, 50, 75, 100 mM NaCl). The used chemicals were fusicoccin (5 μM), gibberellic acid (3 mM), kinetin (0.5 mM), nitrate (20 mM), thiourea (10 mM) and ethephon (10 mM). Seeds were germinated in a growth chamber set at 30/40 °C (12h dark/12 h light), which is the most appropriate temperature for the germination of *C. conglomeratus*. The germination was conducted in 9-cm Petri-dishes containing one disk of Whatman No. 1 filter paper with 10 ml of test solution. Each dish was wrapped with Para film as an added precaution against loss of water by evaporation. The germination was recorded as mentioned above.

2.7. Drought experiment

The impact of drought on seed germination was assessed by using different concentrations of polyethylene glycol 6000 (PEG-6000) that produced different levels of osmotic pressures. Different osmotic pressures (0, -0.2 and -0.5 MPa) were used and those that used for *L. scindicus* were 0, -0.2, -0.5, -0.7 and -1.0 MPa (Michel and Kaufman, 1973). Seeds were germinated at 25°C in continuous light

2.8. Compositions and Quality of Seeds

2.8.1 Collection and Preparation of Samples

Matured seeds of *Cyperus conglomeratus* were collected from different regions of the UAE. The eastern region of the country was represented by Al-Ain region (Zakhir, Manaseer), the western region was represented by two sites in Abu Dhabi (Al Khettem and Al Wathbah) and the northern region of the country was represented by one site in Dubai. Seeds were randomly collected from the whole population to represent the genetic diversity of the population. Seeds were separated from litters by using a series of sieves and dry stored in brown coloured paper bags at room temperature. The seeds from different regions were ground separately and the powder was stored in airtight containers.

2.8.2 Analysis of Samples

The compositional analysis of C. *conglomeratus* seeds was carried out using official standard methods published by the Association of Official Analytical Chemists (AOAC) (2000). Crude fiber, ADF and NDF are analyzed following AOCS official method using ANKROM fiber analyzer.

2.8.3 Moisture

About 5 grams of accurately weighed sample was placed in an oven maintained at 105 ± 2 °C and the change in the sample weight was monitored. When the sample weight was constant, the loss in weight of sample was calculated as percent of moisture in the sample.

2.8.4 Ash

About 2 g of prepared sample, was weighed into a clean dry silica crucible and the organic matter was incinerated in a muffle furnace maintained at 500 °C for 8 hours. The crucible was removed from the furnace and allowed to cool to room temperature. A few drops of deionized water were added and the residual organic matter was oxidized with 2 ml of H_2O_2 by careful heating on a hotplate. The crucible was then returned to the muffle furnace and heated for further 1h. The crucible was cooled in a desiccator, weighed and the total ash was expressed as percentage.

2.8.5 Protein

A known quantity of sample (about 1 gram) was accurately weighed and digested with concentrated sulphuric acid in the presence of a catalyst using the Kjeldahl procedure. Ammonia liberated after neutralizing the acid by alkali was steam distilled into boric acid solution using Kjeltec 2300 automated distillation and titration unit. The amount of ammonia was estimated by the titration using standard sulphuric acid (0.1 M) and the Kjeldahl nitrogen was converted into protein (N x 6.25).

2.8.6 Minerals and Heavy Metals

About 1 gram of sample accurately weighed into a Teflon vessel and treated with highly pure (BDH, Aristar grade) nitric and hydrochloric acids to destroy the organic matter. The Teflon vessel was heated on a hot plate to completely digest the sample. After cooling, the solution was made up to 50 ml in a volumetric flask with deionized water and filtered. Minerals (Na, K, Ca, Mg, Cu, Zn, Fe, P) and heavy metals (Pb and Cd) in the sample

solution were determined using calibrated Inductively Coupled Plasma – Atomic Emission Spectrophotometer (ICP-AES, Varian Vista MPX) at appropriate emission wavelengths.

2.8.7 Total Carbohydrates

Percentage of total carbohydrates were determined by difference (subtracting moisture,fat, protein and ash from one hundred).

2.8.8 Crude Fiber ,Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF):

Crude fiber largely consists of cellulose; ADF is the fraction of undigestible plant material, usually cellulose fiber coated with lignin. As ADF increases, digestibility of a forage usually decreases. NDF is the total cell wall, which is comprised of the ADF fraction plus hemicellulose. NDF values are important in ration formulation because they reflect the amount of forage the animal can consume. As NDF percentages increase, dry matter intake will generally decrease. Crude Fiber, ADF and NDF were analyzed following official AOCS method using ANKROM Fiber Analyzer using the procedure of Van Soest et al. (1991).

2.8.9 Extraction methods

Soxhlet method

A known quantity of sample (about 5 grams accurately weighed), was placed in a thimble and subjected to continuous extraction in an automated Soxhlet Extractor (Soxhtec 2300) using n-hexane as solvent. After extraction, rinsing and solvent recovery cycles the residue (after evaporating the residual solvent) was weighed as fat.

SFE Method

The supercritical fluid technology using CO_2 (99.995% pure), was used for the extraction of oil. The apparatus consisted of a 260 mL capacity syringe pump and controller system (ISCO 260D), and an ISCO series 2000 SCF extraction system (SFX 220) consisting of a

dual chamber extraction module with two 10 mL stainless steel vessels. Temperature and pressure within the vessels were measured and independently adjusted. The 10 mL stainless steel cell (diameter 1.5 cm) was filled with a known quantity (about 5 g) of ground seeds. The cell was pressurized and heated to the desired pressure and temperature and kept for about 15 min to reach equilibrium. A fixed volume of CO_2 (200 mL) was passed through the cell at a flow rate ranging between 4-5 mL/min. The extract was collected in about 4 mL ethanol after depressurization of the gas. The lines were flushed with 5 mL ethanol to collect remnants of extract in the lines.

2.8.10 Determination of Fatty Acid Profile of C. conglomeratus Oil

About 50 mg of oil was mixed with 2 ml alcoholic potassium hydroxide in a reaction vial. The vial was sealed with a screw cap and teflon septum. The vial was heated in hot oven for 10 min. after cooling, 1 ml boron trifluoride-methanol reagent was added and the vial was reheated for 10-15 min. After cooling to room temperature, 1 ml heptane was added followed by heating for 2 min. The vial was cooled to room temperature, deionized water was added and the separated heptane layer was collected into another vial. The heptane layer was dried with anhydrous Na_2SO_4 and finally transferred into a clean GC vial. The fatty acid profile was analyzed on Varian CP 3800 Gas Chromatograph using FAME column. GC conditions were as follows: Injector type: 1177, Temperature: 240 °C, Split ratio: 1:2 , Carrier gas: He, 1.0 mL/min., Detector: FID- 280 °C, Column: Select FAME (Varian CP 7419, 50 m x 0.25 mm id), Temperature Programming: 80 °C - 2 min. – 5 °C/min – 180 °C - 5 min. 5 °C/min – 240 °C – 5 min. Certified fatty acid methyl esters mixed standard (Supelco FAME Mix C8-C22) was injected to record the retention times and relative areas. Prepared sample solutions were analyzed in duplicate and fatty acid profile of oil was determined by normalization technique.

2.9. Calculations and Statistical Analysis

The rate of germination was estimated using a modified Timson index of germination velocity = $\Sigma G/t$, where G is the percentage of seed germination at 2 day intervals and t is the total germination period (Khan and Ungar 1984). The maximum value possible using this index with the data of the present study was 1000/20=50. The higher the index value, the more rapid was the germination.

A three-way analysis of variance (ANOVA) was carried out to demonstrate the effects of the main factors (maternal habitat, salinity or storage and both light and temperature of incubation) and their interactions on the final germination percentage as a dependent variable. Two-way ANOVAs were performed to evaluate the effect of salinity and temperature on germination rate and the effect of light and temperature on germination percentage of non-saline treated seeds. Two-way ANOVA was also carried out to test the effects of dormancy regulating chemicals and salinity and their interaction on both final germination percentage and germination rate. The same test was used to assess the impact of salinity and temperature and their interaction on recovery germination, after the transfer of the ungerminated seeds in the different salinities to distilled water. In order to evaluate the impact of dormancy regulating chemicals on innate dormancy, one way ANOVA was performed on final germination and germination rate of the non-saline treated seeds. One-way ANOVAs were also done when significant interaction between factors were found. Tukey test (Honestly significant differences, HSD) was used to estimate least significant range between means. The germination percentages were arcsine transformed to meet the assumptions of ANOVA. The transformation improved normality of distribution of data. All statistical methods were performed using SYSTAT, version 11.0.

CHAPTER 3

RESULTS

3. Results

3.1. Effects of maternal habitat

3.1.1 Effect of final germination

The effects of maternal habitat, temperature and light of incubation on final germination percentage of *cyperus conglomeratus* seeds was significant ($P<0.001$, Table 4). The final germination was significantly greater for seeds collected from Al Wathbah (43.2%), Al Khattem (41.7%) and Dubai (41.9%), compared to the other habitats. In addition, seeds of Liwa attained significantly lower germination (22.5%), compared to those of the other habitats. Germination in light (38.5%) was significantly greater than in dark (31.0%). Very few seeds germinated at constant 30 °C temperature (2.9%), compared to all other fluctuating temperatures. Germination at 30/40 °C (63.6%) was significantly greater than at 25/35 °C (47.9%) and both attained significantly greater values than at 20/30 °C (22.7%).

The interaction between maternal habitat and temperature on the final germination was highly significant ($P<0.001$, Table 4). This indicates that temperature requirement for germination depended on maternal habitat. For example, whereas germination at 30/40 °C was significantly greater than at 25/30 °C for seeds collected from Dubai, industrial area of Al-Ain and Manasir, it did not differ from each other at the other for seeds of the other habitats. In addition, germination at 20/30 °C was significantly greater than at the other two higher temperatures (25/35 and 30/40 °C), but this was more pronounced for seeds of Manasir and Dubai (Figure 3)

The ANOVA also showed a significant interaction effect between the light and maternal habitat ($P<0.001$, Table 4). Germination in light was greater than in dark for seeds of all maternal habitats, except for seeds of Liwa, where the reverse was true. Light germination

was greater than dark germination by 29%, 28%, 23%.21.8%, 21.1% and 19.7 for seeds of Manasr, Dubai, industrial area of Al Ain, AlWathbah, Zakhir and Al Khetem, respectively, but dark germination was greater than light germination by 16.7% for seeds of Liwa (Table 5, Figure 4)

The effects of the interaction between light and temperature on final germination was also highly significant ($P<0.001$, Table 4). At fluctuating temperatures, the difference between light and dark germination was insignificantly at both the lowest and the highest temperatures, but light germination was significantly greater than dark germination at moderate temperatures. Light germination was greater than dark germination by 23% at 25/35 °C, but only by 9.6% and 10.8% at 20/30 °C and 30/40 °C, respectively (Table 5, Figure 5).

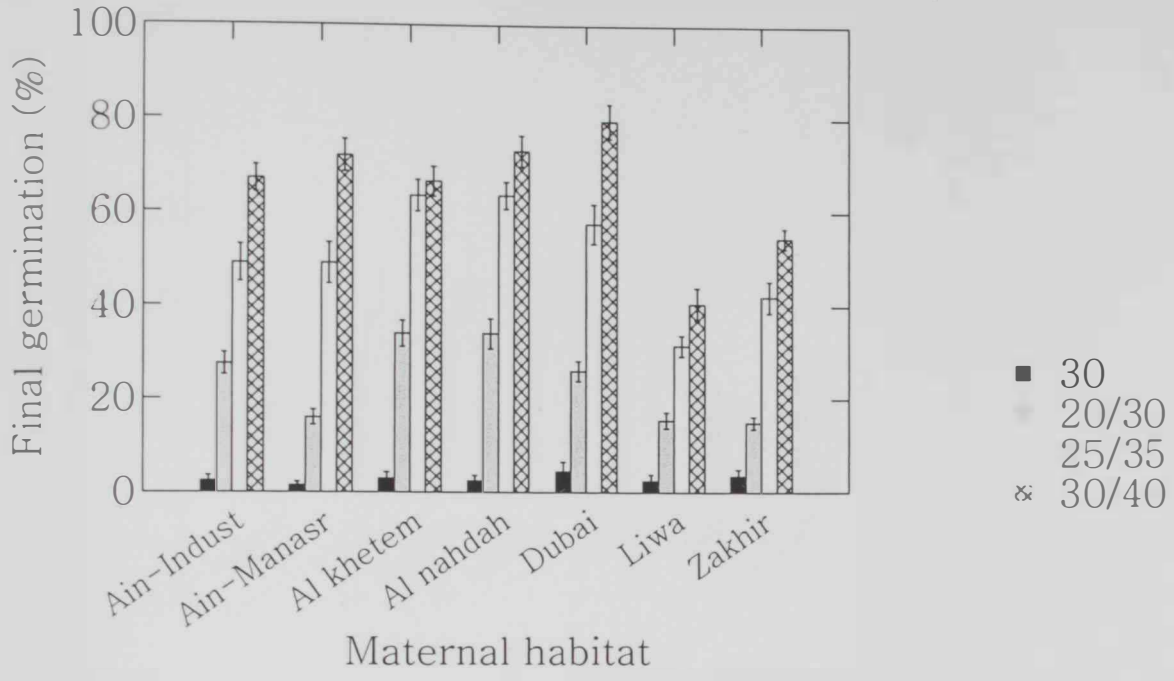
The ANOVA also showed a significant interaction effect between maternal habitat, and light and temperature of the incubation ($P<0.001$, Table 4). Germination in light was greater than it in dark at 30/40 °C for seeds collected from the industrial area of Al Ain and Dubai, but the difference was insignificant for seeds of the other habitats. At 20/30 °C, germination in light was significantly greater than in dark for seeds of all habitats, except that for seeds of Liwa, Al Khetem and Al Wathbah, where the difference was insignificant (Table 5 and Figure 6).

Table 4: Three-way ANOVA testing the effects of maternal habitat, temperature and light of incubation on final germination percentage of *Cyprus conglomeratus* seeds.

Source	df	Mean-Square	F-ratio	P
Maternal habitat (M)	6	0.266	53.191	<0.001
Temperature (T)	3	5.304	1058.863	<0.001
Light (L)	1	0.358	71.501	<0.001
M*T	18	0.061	12.262	<0.001
M*L	6	0.027	5.354	<0.001
T*L	3	0.033	6.580	<0.001
M*T*L	18	0.009	1.745	<0.05
Error	168	0.005		

Figure 3: Effects of maternal habitat and temperature of incubation on final germination percentage and germination rate (mean ± standard error) of *Cyprus conglomeratus* seeds

(a) Final germination



(b) Germination rate

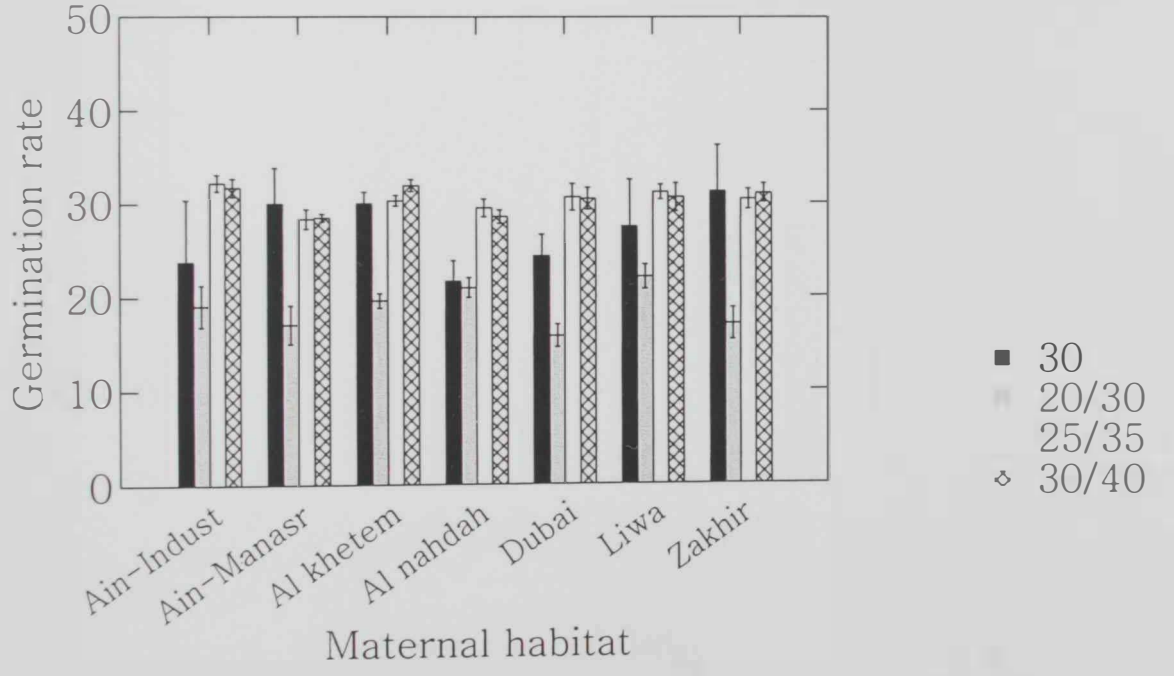
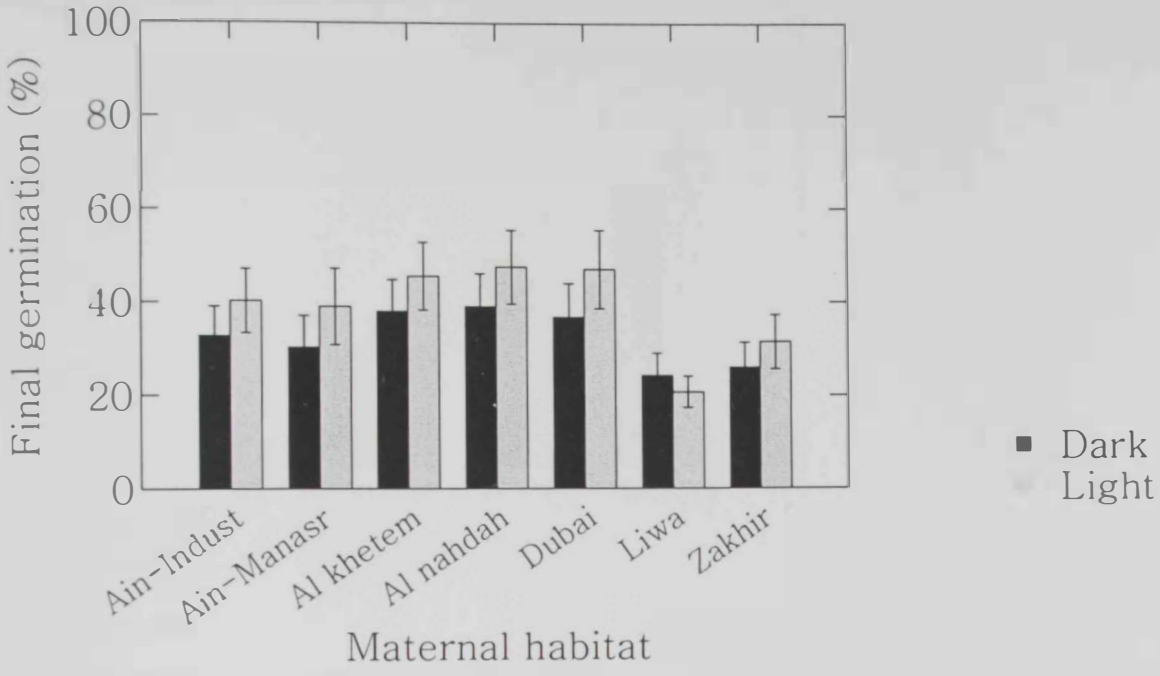


Figure 4: Effects of maternal habitat and light of incubation on final germination percentage and germination rate (mean ± standard error) of *Cyprus conglomeratus* seeds

(a) Final germination



(b)Germination rate

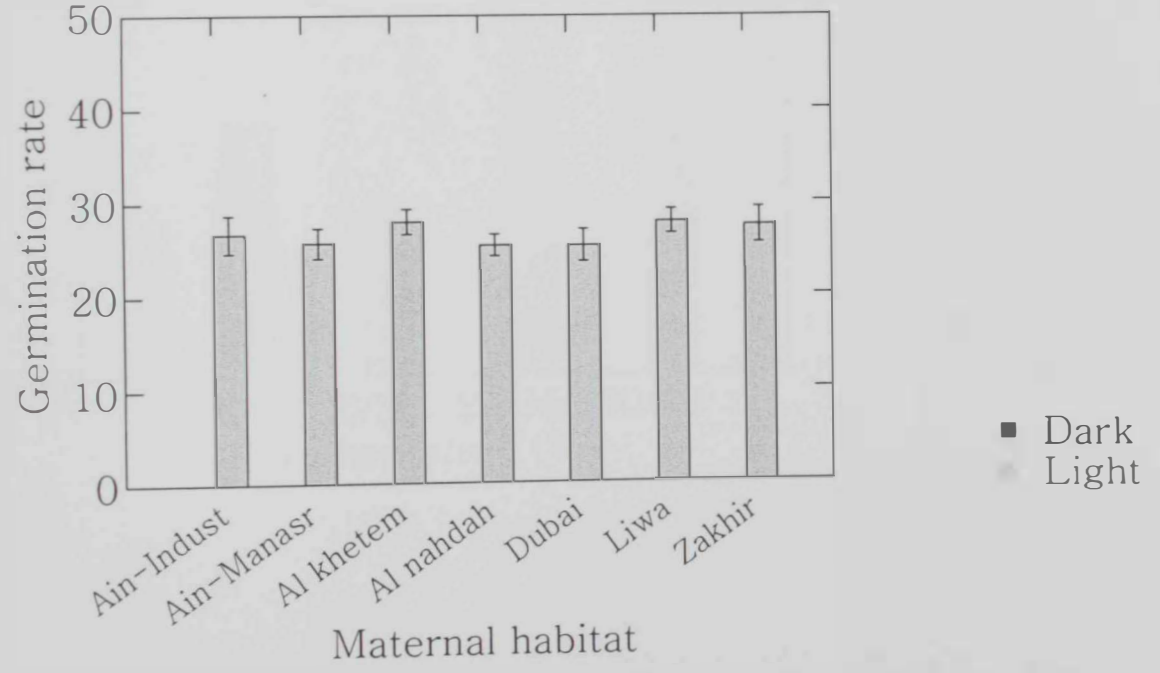
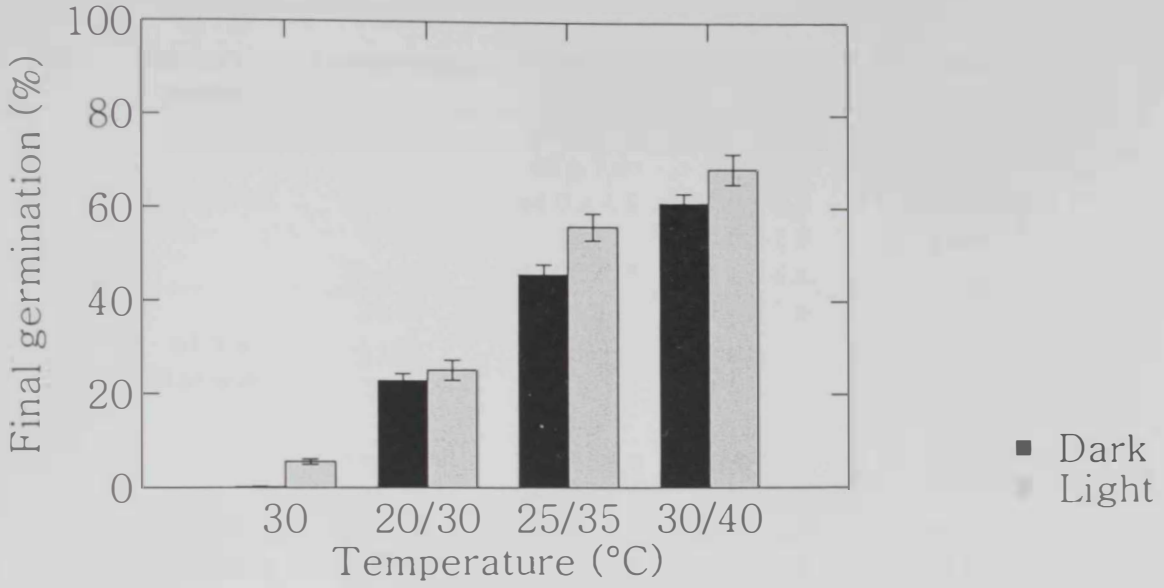


Figure 5: Effects light and temperature of incubation on final germination percentage and germination rate (mean ± standard error) of *Cyprus conglomeratus* seeds
(a) Final germination



(b)Germination rate

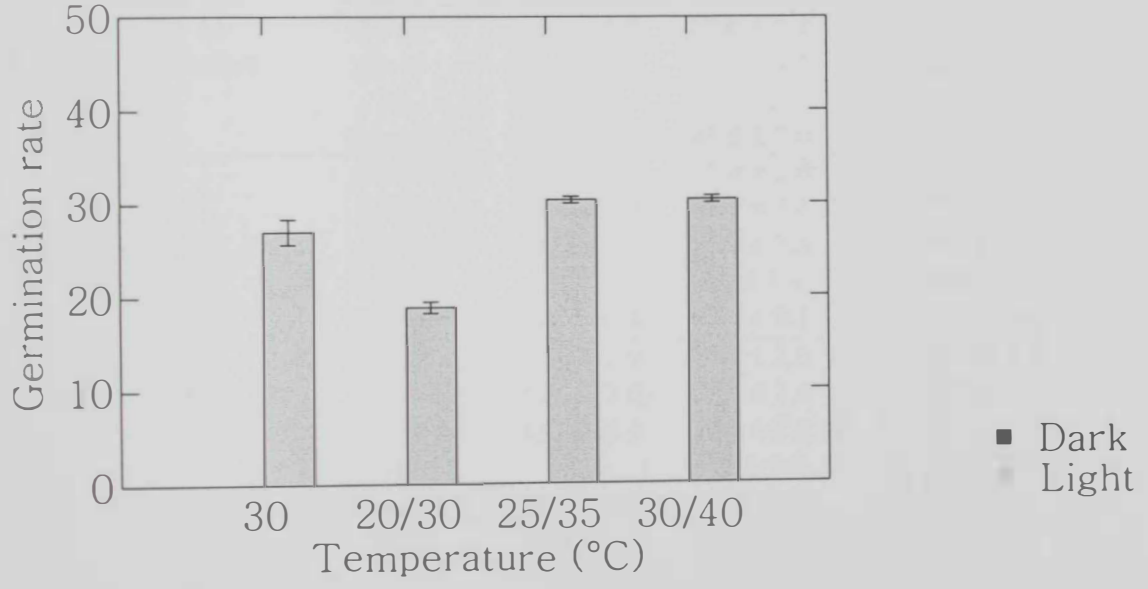


Table 5: Effects of maternal habitat, temperature and light of incubation on final germination percentage (mean ± standard error) of *Cyprus conglomeratus* seeds

Maternal habitat	Temperature	Dark	Light	Overall
Al Ain Industrial	20/30	27 ± 4.1	28 ± 2.3	27.5 ± 2.2
	25/35	40 ± 1.6	58 ± 2.6	49.0 ± 3.7
	30/40	64.0 ± 4.6	70.0 ± 2.6	67.0 ± 2.7
	30	0.0	50.0 ± 1.0	2.5 ± 1.1
	Overall	**32.7 ± 6.1**	**40.2 ± 6.6**	.....
Al Ain Manaseer	20/30	14 ± 1.2	18.0 ± 2.6	16.0 ± 1.5
	25/35	41.0 ± 2.5	57.0 ± 5.3	49.0 ± 4.1
	30/40	66.0 ± 3.5	78.0 ± 3.5	72.0 ± 0.7
	30	0.0	3.0 ± 1.0	1.5 ± 3.2
	Overall	**30.25 ±6.6**	**39 ± 7.9**	.....
Al Wathbah	20/30	29.0 ± 3.0	39.0 ± 4.1	34 ± 3.0
	25/35	59.0 ± 2.5	68.0 ± 3.7	63.5 ± 2.7
	30/40	67.0 ± 3.0	79.0 ± 3.4	73.0 ± 3.1
	30	1.0 ± 1.0	4.0 ± 1.6	2.5 ± 1.1
	Overall	**39.0 ± 6.8**	**47.5 ± 7.6**	.....
Al Khetem	20/30	31.0 ± 2.5	37.0 ± 4.4	34 ± 2.6
	25/35	60.0 ± 4.3	67.0 ± 4.4	63.5 ± 3.2
	30/40	61.0 ± 2.5	72.0 ± 3.7	66.5 ± 2.9
	30	0.0	6.0 ± 1.2	3.0 ± 1.3
	Overall	**38.0 ± 6.6**	**45.5 ± 7.0**	.....
Dubai	20/30	26.0 ± 3.5	26.0 ± 2.6	57.5 ± 3.9
	25/35	49.0 ± 3.0	66.0 ± 3.8	79.5 ± 3.4
	30/40	72.0 ± 1.6	87.0 ± 3.8	4.5 ± 1.9
	30	0.0	9.0 ± 1.9	26.0 ± 2.0
	Overall	**36.75 ±7.0**	**47.0 ± 8.1**	.....
liwa	20/30	17.0 ± 1.9	14.0 ± 2.6	15.5 ± 1.6
	25/35	35.0 ± 3.0	28.0 ± 1.6	31.5 ± 2.1
	30/40	45.0 ± 5.3	36.0 ± 2.8	40.5 ± 3.2
	30	0.0	5.0 ± 1.9	2.5 ± 1.3
	Overall	**24.25 ±4.7**	**20.75 ±3.3**	.....
Zakhir	20/30	16.0 ± 1.6	14.0 ± 2.0	15.0 ± 1.3
	25/35	36.0 ± 2.8	48.0 ± 3.7	42.0 ± 3.1
	30/40	52.0 ± 2.8	57.0 ± 2.5	54.5 ± 2.0
	30	0.0	7.0 ± 1.0	3.5 ± 1.4
	Overall	**26.0 ± 5.2**	**31.5 ± 5.6**	.....

Figure 6: Effects of maternal habitat, temperature and light of incubation on final germination percentage and germination rate index (mean ± standard error) of *Cyprus conglomeratus* seeds. Dark bars = dark germination and light bars = light germination

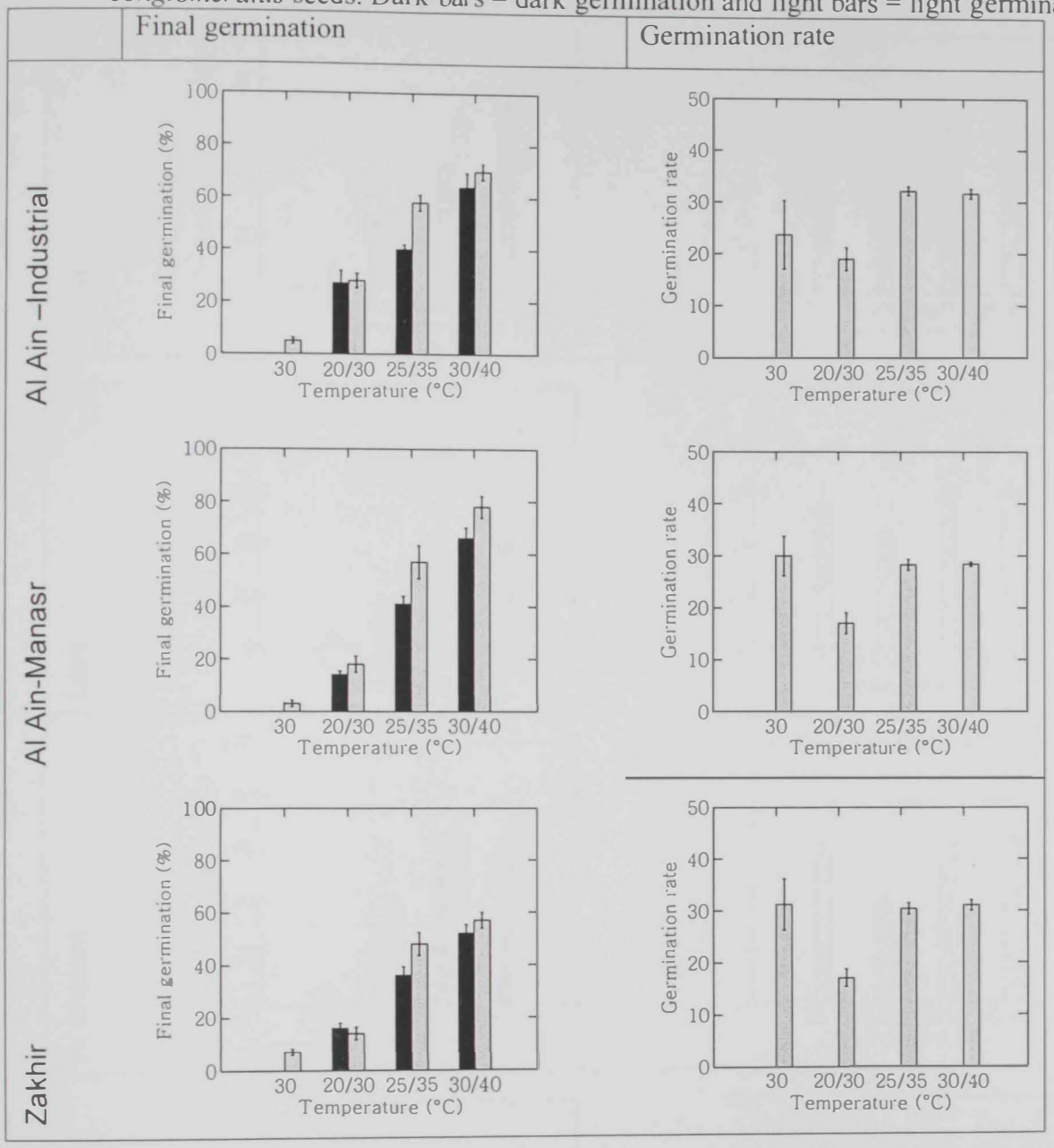
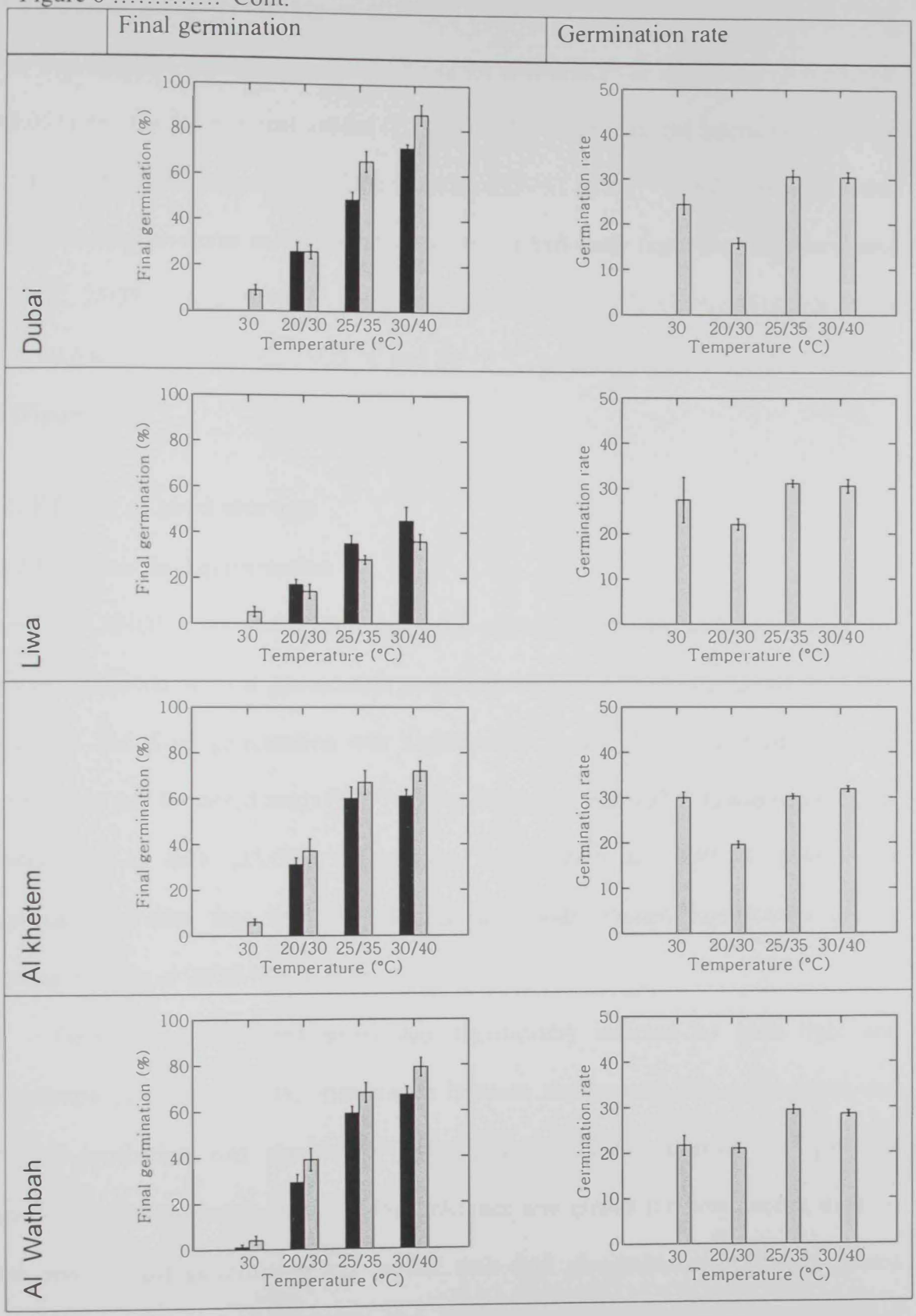


Figure 6 ..... Cont.



3.1.2 Effect of germination rate

Two-way ANOVA showed significant effects for temperature of incubation ($F = 58.510$, $P<0.001$), but not for maternal habitat ($F = 1.372$, $P = 0.236$) and the interaction between maternal habitat and temperature of the incubation ($F = 1.646$, $P = 0.068$), on germination rate of *C. conglomeratus* seeds. Germination was significantly faster for seeds incubated at 30 °C, 25/35 °C and 30/40 °C, compared to that at 20/30 °C. Germination rates were 27.2, 30.6 and 30.7 at 30 °C, 25/35 °C and 30/40 °C, respectively, but was 19.5 at 20/30 °C (Figures 3-5).

3.2. Effects of seed storage

3.2.1 Effect on final germination

Three-way ANOVA showed highly significant effects for storage, and temperature and light of incubation on final germination percentage of *C. conglomeratus* seeds ($P<0.001$, Table 6). The final germination was significantly greater for fresh seeds (34.6%), compared to that for stored seeds (28.7%). Germination in light (37.8%) was significantly greater than in dark (25.6%). In addition, germination at 30/40 °C (64%) was significantly greater than at 25/35 (45.5%) and both attained significantly greater germination than at 20/30 °C (14.8%).

Germination of stored seeds was significantly affected by both light and temperature of incubation; the interactions between storage and both temperature and light of incubation was significant ($P<0.05$, Table 6). Germination in light was significantly greater than in dark, but the difference was greater for stored seeds, than for fresh seeds. Light germination was greater than dark germination by 73% for stored seeds, but by 29% for fresh seeds. Similarly, germination was low in stored, compared to fresh seeds, so the reduction was greater at higher than at lower temperatures.

Germination of fresh seeds was greater than that of stored seeds by 29% at 30/40 °C, but by 16% and 18% at 25/35 and 20/30 °C, respectively (Figure 7).

The interactive effect between light and temperature on final germination was also highly significant ($P<0.001$, Table 6). Whereas light germination was greater than dark germination at all fluctuating temperatures, the difference was significant at higher temperatures. The light germination was greater than dark germination by 49% and 42% at 25/35 °C and 30/40 °C, respectively, but by 27% at 20/30 °C (Figure 7).

3.2.2 Effect on germination rate

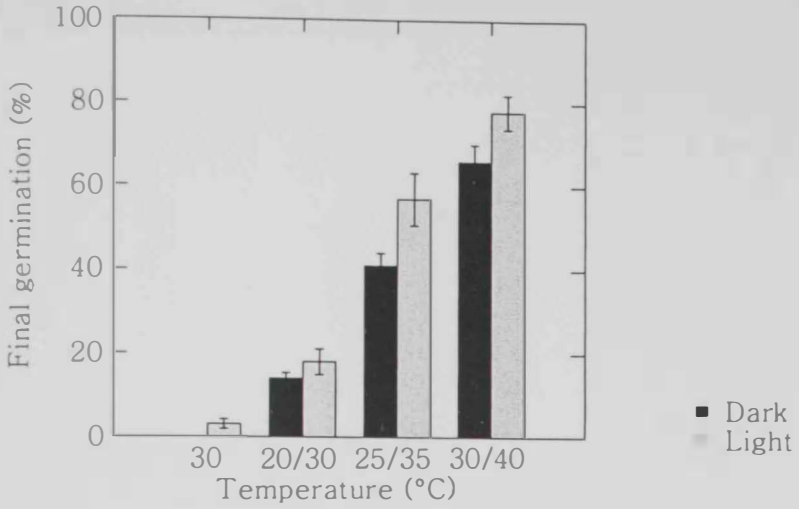
Two-way ANOVA showed significant effects for storage ($F = 10.47$, $P= 0.004$) and the interaction between storage and temperature ($F = 3.266$, $P= 0.04$) of incubation and highly significant effect for temperature of incubation ($F = 18.70$, $P<0.001$) on germination rate of *C. conglomeratus* seeds. Germination was significantly faster for seeds incubated at 30 °C, 25/35 °C and 30/40 °C, compared to that at 20/30 °C. Germination rates were 29.3, 30.5 and 30.7 at 30 °C, 25/35 °C and 30/40 °C, respectively, but was 20.7 at 20/30 °C (Figure 8). Unlike the trend observed for final germination, germination rate of stored seeds (29.7) was significantly faster than for fresh seeds (25.7) (Figure 8).

Table 6: Three-way ANOVA testing the effect of storage, temperature and light of incubation on final germination percentage of *Cyprus conglomeratus* seeds collected from Ain-Manasr area, Al-Ain. Ns: insignificantly differ at P = 0.05

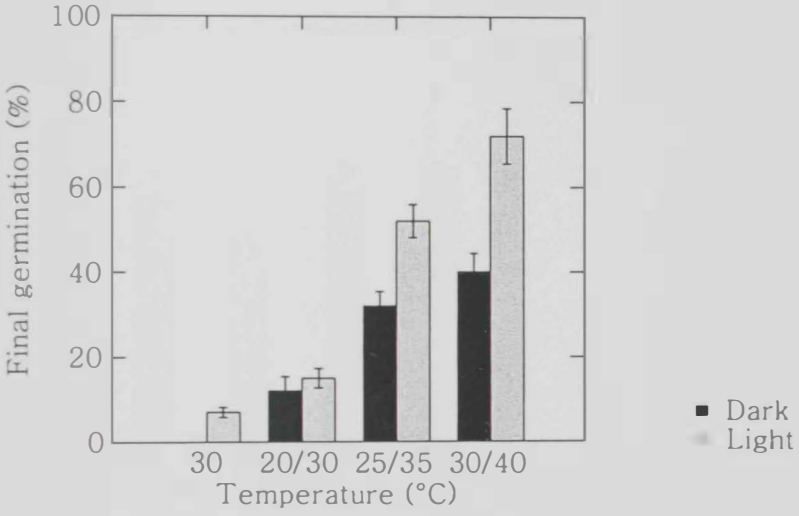
Source	Df	Mean-Square	F-ratio	P
Storage (S)	1	0.081	14.727	<0.001
Temperature (T)	3	1.565	283.867	<0.001
Light (L)	1	0.335	60.710	<0.001
S*T	3	0.036	6.549	<0.001
S*L	1	0.031	5.167	<0.05
T*L	3	0.060	10.904	<0.001
S*T*L	3	0.011	1.937	Ns
Error	48	0.006		

Figure 7: Effects of storage, light and temperature of incubation on final germination percentage (mean ± standard error) of *Cyprus conglomeratus* seeds

(a) Fresh seeds



(b)Stored seeds



(c) Overall fresh and stored seeds

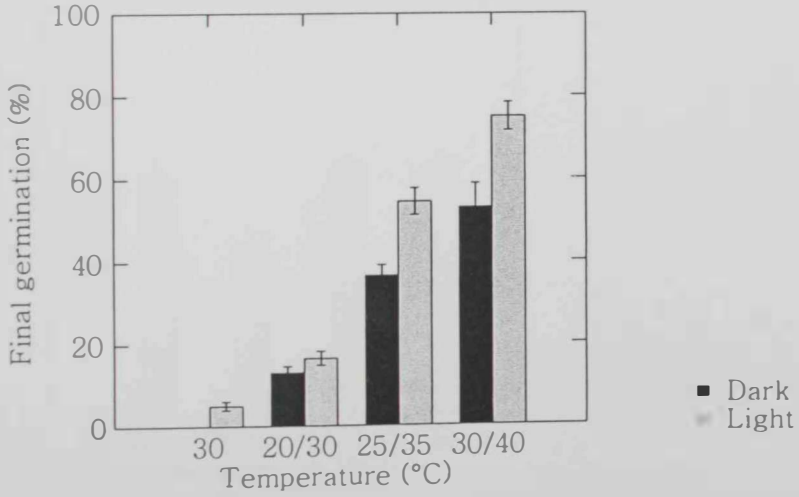
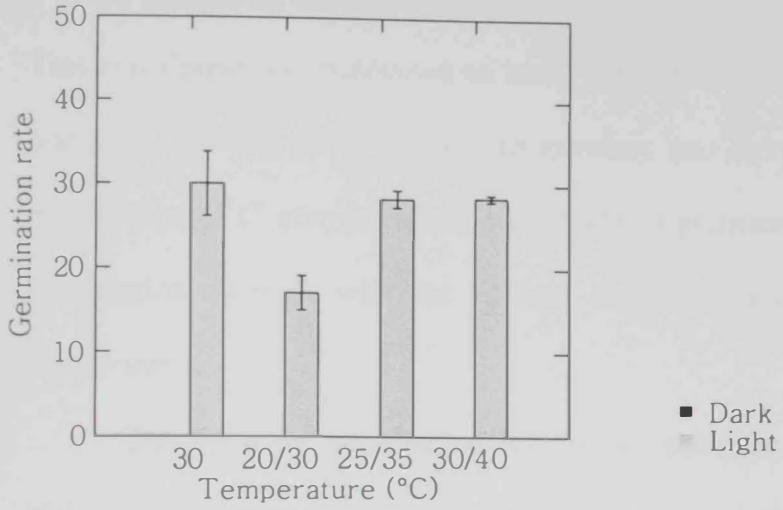
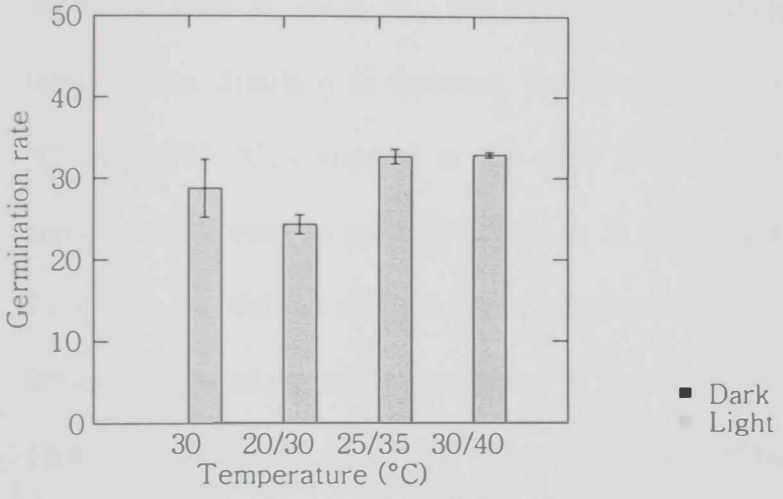


Figure 8: Effects storage, and light and temperature of incubation on germination rate (mean ± standard error) of *Cyprus conglomeratus* seeds

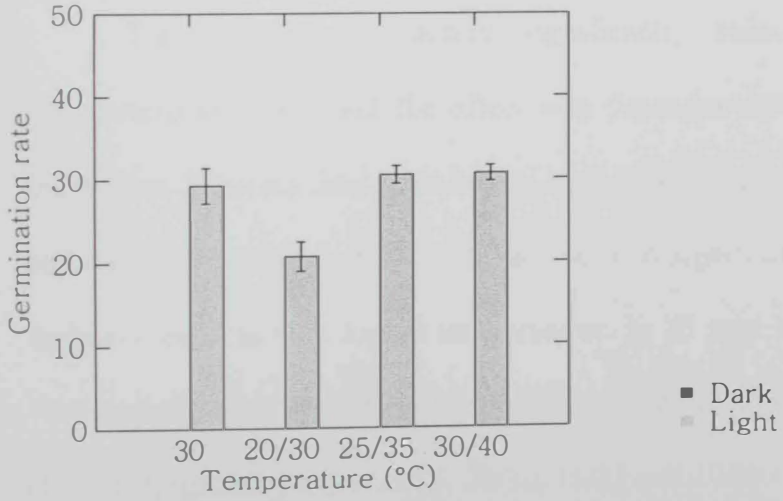
(a) Fresh seeds



(b)Stored seeds



(c) Overall fresh and stored seeds



3.3. Effects of Salinity, Temperature and Light on Final Germination

3.3.1 Effects on final germination

This experiment was conducted on seeds collected from Manaser, Al-Ain, and stored for one year. The effects of salinity, temperature and light and their interactions on final germination of *C. conglomeratus* were highly significant ($P<0.001$, Table 7). Generally, germination decrease with the increase in salinity and increase with the increase in temperatures.

The optimum germination for the non-saline treated seeds was in light and at highest temperature (30/40 °C). In light, final germination was significantly greater at 30/40 °C than at 25/35 °C, and both were significantly greater than at the lower temperatures. Similarly in darkness, final germination was significantly greater at 30/40 °C and 25/35 °C, compared to the other lower temperatures. Final germination was significantly greater in light, compared to in darkness at higher temperature (30/40 and 25/35 °C), but did not differ at lower temperatures (20/30, 15/25 and 10/20 °C). About 80% and 50% of the seeds germinated in light at 30/40 and 25/35 °C, respectively, but 18.8% at both 15/25 and 20/30 °C. In dark, only 35%, 37.5%, 21.3%, 15% and 2.5% germinated at 30/40, 25/35, 20/30, 15/25 and 10/20, respectively (Figure 9, Table 8).

The increase in salinity significantly reduced final germination of *C. conglomeratus* seeds, and the effect was dependant on temperature and light of seed incubation. Whereas final germination in light was significantly reduced in 25 mM NaCl and almost inhibited in 50 – 100 mM NaCl, it significantly increased or not affected in darkness, especially at higher temperatures. In 25 mM NaCl, final germination at 30/40 °C in light (36.3%) was significantly greater than at all other lower temperatures in light (17.0, 6.3, 6.3 and 0.0 for 25/35, 20/30, 15/25 and 10/20

Table 7: Three-way ANOVA showing the effect of salinity, temperature and light of incubation on final germination of *Cyprus conglomeratus* seeds

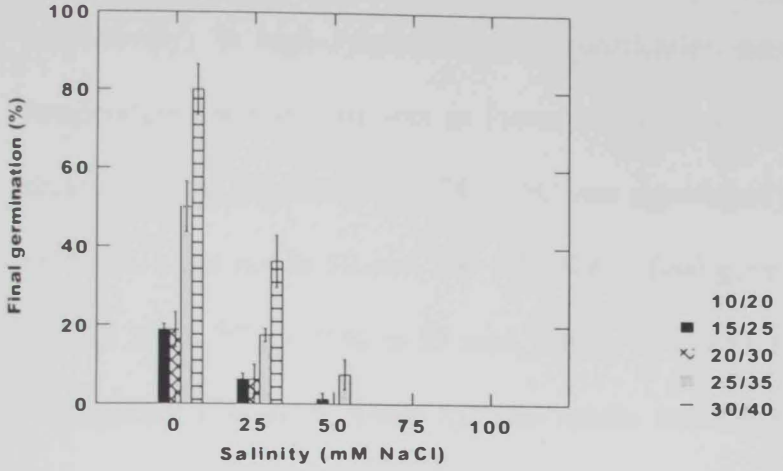
Source	df	Mean-Square	F-ratio	P
Salinity (S)	4	0.503	129.838	<0.001
Temperature (T)	4	0.608	156.860	<0.001
Light (L)	1	0.266	68.677	<0.001
S*T	16	0.069	17.779	<0.001
S*L	4	0.159	41.145	<0.001
T*L	4	0.073	18.904	<0.001
S*T*L	16	0.058	14.947	<0.001
Error	150	0.004		

Table 8: Effect of salinity, temperature, light and their interactions on final germination (mean ± standard error) of *Cyprus conglomeratus*

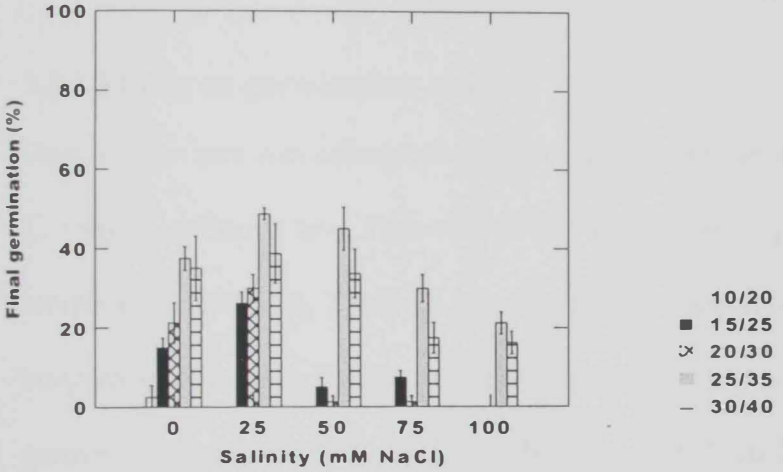
Salinity	Temp.	Temperature					
		10/20	15/25	20/30	25/25	30/40	Overall
0	Dark	2.5 ± 2.5	15.0 ± 2.04	21.3 ± 4.3	37.5 ± 2.50	35.0 ± 6.77	22.3 ± 3.4
	light	0.0 ± 0.0	18.8 ± 1.3	18.8 ± 3.8	50.0 ± 5.40	80.0 ± 5.40	33.5 ± 6.7
	overall	1.3 ± 1.3	16.9 ± 1.32	20.0 ± 2.7	43.8 ± 3.6	57.5 ± 9.4	27.9±3.8
25	Dark	0.0 ± 0.0	26.3 ± 2.4	30.0 ± 2.9	48.8 ± 1.3	38.8 ± 6.3	28.8 ± 3.9
	light	0.0 ± 0.0	6.3 ± 1.3	6.3 ± 3.2	17.5 ± 1.44	36.25 ± 5.54	13.3 ± 3.2
	overall	0.0 ± 0.0	16.3 ± 3.9	18.1 ± 4.9	33.1 ± 5.9	37.5 ± 3.9	21.0±2.7
50	Dark	0.0 ± 0.0	5.00 ± 2.04	1.25 ±1.25	45.0 ± 4.6	33.8 ± 5.2	17.0 ± 4.5
	light	0.0 ± 0.0	1.25 ± 1.25	0.0 ± 0.0	7.5 ± 3.2	0.0 ± 0.0	1.8 ± 0.9
	overall	0.0 ± 0.0	3.1 ± 1.3	0.6 ± 0.6	26.3 ± 7.5	16.9 ± 6.8	9.3±2.6
75	Dark	0.0 ± 0.0	7.5 ± 1.4	1.3 ± 1.3	30.0 ± 2.9	17.5 ± 3.2	11.3 ± 2.7
	light	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	overall	0.0 ± 0.0	3.7 ± 1.6	0.6 ± 0.6	15.0 ± 5.8	8.8 ± 3.6	5.6±1.6
100	Dark	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	21.3 ± 2.4	16.3 ± 2.4	7.5 ± 2.2
	light	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	overall	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.6 ± 4.1	8.1 ± 3.2	3.7±1.3
Overall	Dark	0.5 ± 0.5	10.8 ± 2.2	10.8 ± 3.0	36.5 ± 2.6	28.3 ± 2.9	9.7±1.9
	light	0.0 ± 0.0	5.3 ± 1.7	5.0 ± 1.9	15.0 ± 4.4	23.3 ± 7.4	17.4±1.7
	overall	0.25±0.25	8.0±1.4	25.8±3.1	25.8±3.9	7.9±1.8	.....

Figure 9: Effect of salinity, temperature and light of incubation on final germination (mean ± standard error) of *Cyprus conglomeratus* seeds.

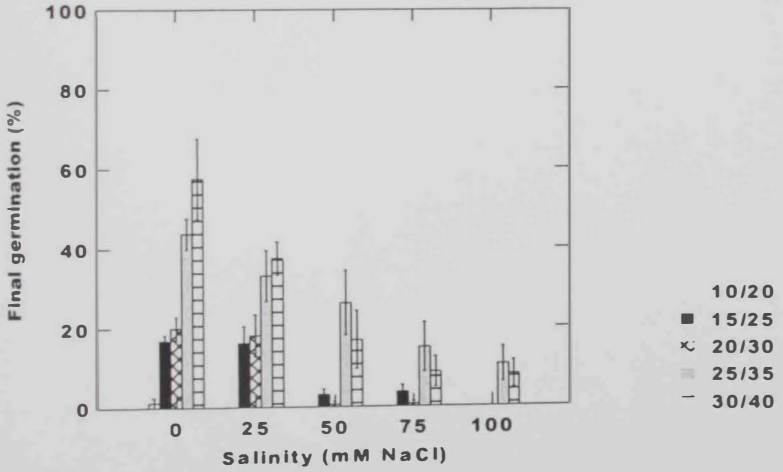
(a) Light germination



(b) Dark germination



(c) Overall light and dark germination



°C, respectively), but not differed from that in darkness at all temperatures, except 10/20 °C (38.8%, 48.8%, 30.0%, 26.3% and 0.0% for 30/40, 25/35, 20/30, 15/25 and 10/20 °C, respectively). In higher salinities, final germination was almost inhibited in light at all temperatures and in darkness at lower temperatures (20/30, 15/25 and 10/20 °C). In darkness, final germination at 25/35 °C was significantly greater than at 30/40 °C in 75 mM NaCl, but not in 50 and 100 mM NaCl; final germination at 30/40 °C was greater than at 25/35 °C by 71% in 75 mM, but by 33% and 31.2% in 50 and 100 mM NaCl, respectively (Figure 9, Table 8). The results indicate that salinity tolerance in higher salinity levels was greatest at both higher temperatures in darkness.

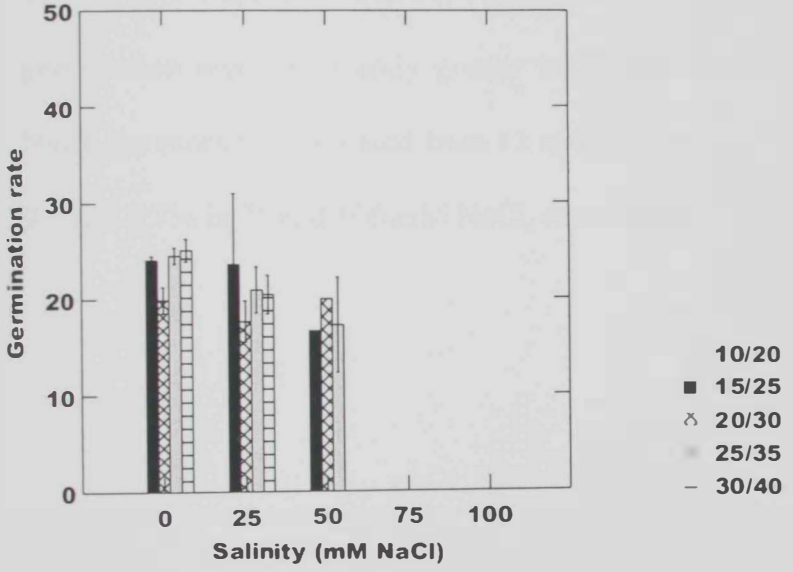
3.3.2 Effects on germination rate

Germination rate was calculated for seeds germinated in light. Generally germination rate of *C. conglomeratus* is low. Two-way ANOVA indicated significant effects for salinity and temperature ($P<0.001$, Table 9), but not their interaction on germination rate of *C. conglomeratus* seeds. Unlike final germination, there was no significant difference between germination rates at 30/40, 25/35, 20/30 and 15/25 °C in 0 and 25 mM NaCl and between 30/40, 25/35 and 20/30 °C in 50 mM NaCl (Figure 10).

Table 9: Two-way ANOVA showing the effect of salinity and temperature of incubation on germination rate of *Cyprus conglomeratus* seeds

Source	Df	Mean-Square	F-ratio	P
Salinity (S)	4	0.965	29.154	<0.001
Temperature (T)	4	1.534	46.357	<0.001
S*T	16	0.052	1.576	Ns
Error	75	0.033		

Figure 10: Effect of salinity and temperature of incubation on germination rate (mean ± standard error) of *Cyprus conglomeratus* seeds.



3.4. Interaction Effects of Salinity and Temperature on Recovery Germination Percentage

Ungerminated seeds in the different salinities were transferred to distilled water to test their ability to recovery germination in light at different temperatures. The effects of salinity and temperature and their interaction on recovery germination were highly significant ($P<0.001$, Table 10). In all salinities, no seeds recovered at the lower temperatures (10/25 and 15/25 °C) and recovery germination percentage was significantly greater at 30/40 °C than 25/35 °C. At 30/40 °C, there was no significant difference between recovery germination in 50, 75 and 100 mM NaCl and all attained significantly lower values than at 25 mM NaCl; the recovery decreased from 43.5% in 25 mM NaCl to 27.1%, 31.3% and 29.4% in 50, 75 and 100 mM NaCl, respectively. At 25/35, recovery germination was significantly greater in 25 and 50 mM NaCl, than in 75 and 100 mM NaCl; the recovery decreased from 12 and 14.8% in 25 and 50 mM NaCl, respectively to 9.7 and 6.7% in 75 and 100 mM NaCl, respectively (Table 11 and Figure 11).

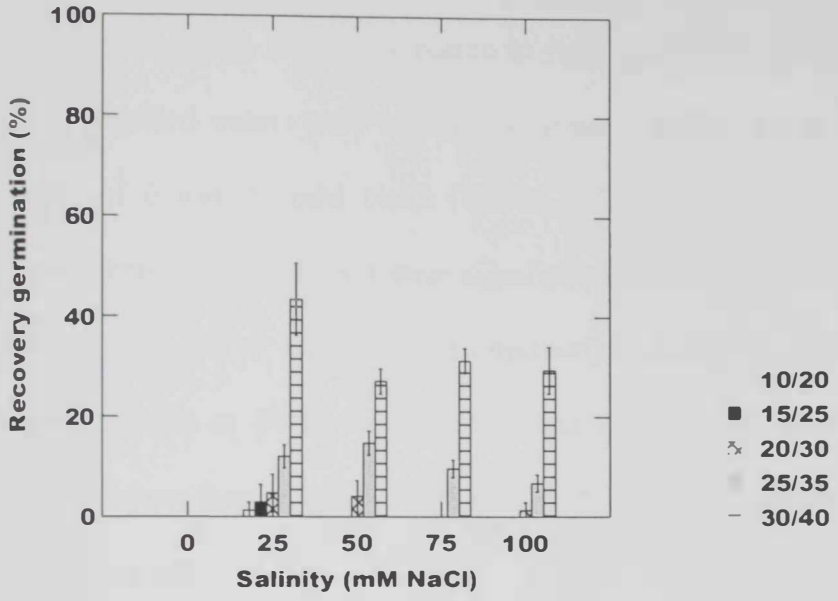
Table 10: Two-way ANOVA showing the effect of salinity and temperature of incubation on recovery germination of *Cyprus conglomeratus* seeds

Source	Df	Mean-Square	F-ratio	P
Salinity (S)	4	0.047	25.764	<0.001
Temperature (T)	4	0.254	140.277	<0.001
S*T	16	0.020	10.885	<0.001
Error	75	0.002		

Table 11: Effect of salinity, temperature, and light on recovery germination percentage (mean ± standard error) of *Cyprus conglomeratus* seeds previously imbibed in various concentrations of NaCl and then transferred to distilled water

Salinity	Temperature					Overall
	10/20	15/25	20/30	25/35	30/40	
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
25	1.3 ±1.3	2.9 ±2.9	4.7 ± 3.2	12.0 ± 1.9	43.5 ± 6.0	7.5 ± 2.7
50	0.0 ± 0.0	0.0 ± 0.0	4.1 ± 2.7	14.8 ± 2.0	27.1 ±2.5	12.9 ±3.9
75	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.7 ± 1.5	31.3 ±2.1	9.2 ± 2.5
100	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.3	6.7 ± 1.5	29.4 ± 3.9	8.2 ± 2.8
Overall	0.3 ±0.3	0.6 ±0.6	2.0 ± 0.9	8.6 ± 1.3	26.2 ± 3.6	.....

Figure 11: Effect of salinity and temperature of incubation on recovery germination (mean ± standard error) of *Cyprus conglomeratus* seeds.



3.5. Interaction Effects of Salinity and Temperature on Total Germination

Two-way ANOVA indicated that the effects of the main factors (salinity and temperature) and their interactions on total germination in light (germination during salt treatment + recovery in distilled water) were highly significant (<0.001, Table 12). Generally, total germination of 0 and 25 mM NaCl (33.5% and 26.2%, respectively) did not differ significantly from each other, but were significantly greater than that in 50, 75 and 100 mM NaCl (11%, 8.2% and 7.5%, respectively). Similarly, total germination was significantly greater at 30/40 °C (49.5%) than at 25/35 °C (23.6%) and both were significantly greater than at 20/30, 15/25 and 10/20 °C (7%, 5.8% and 0.3%, respectively. Table 13, Figure 12).

The interaction between salinity and temperature was significant (P<0.001) indicating that the salinity tolerance during germination was dependant on temperature of incubation. Total germination at 30/40 °C was significantly greater than at 25/35 °C in all salinities, except in 50 mM NaCl (Table 13, Figure 12).

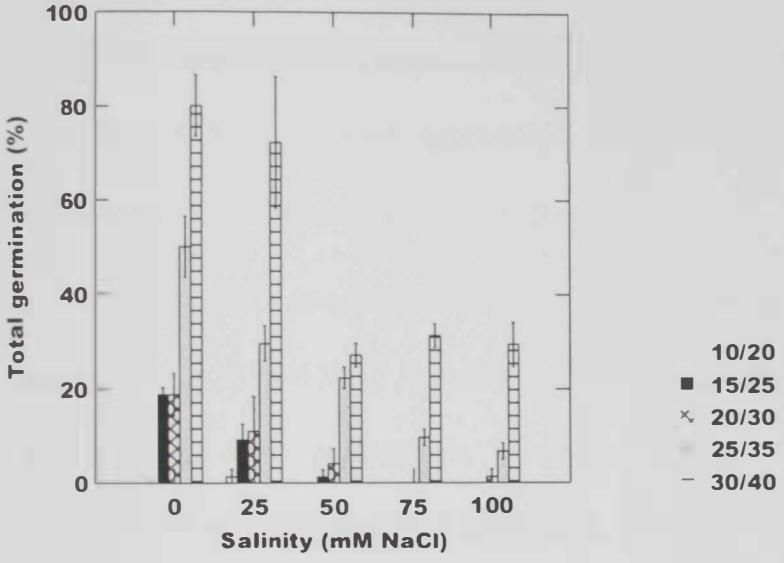
Table 12: Two-way ANOVA showing the effect of salinity and temperature of incubation on total germination percentage (germination during salt treatment + recovery in distilled water) of *Cyprus conglomeratus* seeds

Source	Df	Mean-Square	F-ratio	P
Salinity (S)	4	0.347	75.047	<0.001
Temperature (T)	4	0.650	140.469	<0.001
S*T	16	0.065	14.063	<0.001
Error	75	0.005		

Table 13: Effect of salinity, temperature, and light on total germination percentage (mean ± standard error) of *Cyprus conglomeratus* seeds

Salinity	Temperature					Overall
	10/20	15/25	20/30	25/35	30/40	.....
0	0.0 ± 0.0	18.8 ± 1.3	18.8 ± 3.8	50.0 ± 5.4	80.0 ± 5.4	33.5 ± 6.7
25	1.3 ±1.3	8.8 ± 2.4	10.0 ± 5.4	26.3 ± 2.4	55.0 ± 5.4	6.0 ± 2.0
50	0.0 ± 0.0	1.3 ± 1.3	3.8 ± 2.4	23.8 ± 3.8	27.1 ± 2.1	20.3 ± 4.7
75	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.8 ± 1.3	23.8 ± 1.3	10.0 ± 2.5
100	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.3	6.3 ±1.3	22.5 ± 2.5	6.5 ± 2.2
Overall	0.3 ± 0.3	5.8 ± 1.8	6.8 ± 2.0	23.0 ± 3.8	40.5 ± 5.6	.....

Figure 12: Effect of salinity and temperature of incubation on total germination (germination during salt treatment + recovery in distilled water, mean ± standard error) of *Cyprus conglomeratus* seeds.



3.6. Effects of dormancy regulating chemicals (DRC) on innate and salinity induced dormancy

3.6.1 Effects on Final Germination

Two way ANOVA showed that the effect of DRC and salinity and their interaction on final germination percentage of *Cyprus conglomeratus* seeds was significant ($P<0.001$, Table 14). Seeds of *C. conglomeratus* germinated in distilled water have little innate dormancy; 84% of the seeds germinated. None of the DRC significantly increased final germination of the seeds (Table 15, Figure 13).

Seeds of *C. conglomeratus* were very sensitive to salinity. Only 36% of the seeds germinated in 25 mM NaCl and none in the higher levels (50 mM NaCl or more). The difference in final germination between different salinity levels was insignificant ($P>0.05$), for seeds treated with fusicoccin, GA3 and kinetin, indicating that these DRC completely alleviated salinity induced dormancy in all salinity levels. The difference in final germination between the different salinities was significant for seeds treated with thiourea and nitrate. Nitrate completely alleviated the germination inhibition occured in 25, 50 and 75 mM NaCl (final germination was 79, 81%, respectively), but partially alleviated it in 100 mM NaCl (final germination was 58%). Thiourea, however, completely alleviated salinity induced dormancy in 25 mM NaCl (final germination was 81%), but partially alleviated it in the higher levels (final germination was 45%, 46% and 34% in 50, 75 and 100 mM NaCl, respectively) (Table 15, Figure 13).

Table 14: Two way ANOVA testing the effect of dormancy regulating chemicals and NaCl concentration on final germination percentage of *Cyprus conglomeratus* seeds

Source	Df	Mean-Square	F-ratio	P
DRC	5	1.546	130.0	<0.001
Salinity	4	0.762	64.1	<0.001
DRC*Salinity	20	0.124	10.6	<0.001
Error	90	0.012		

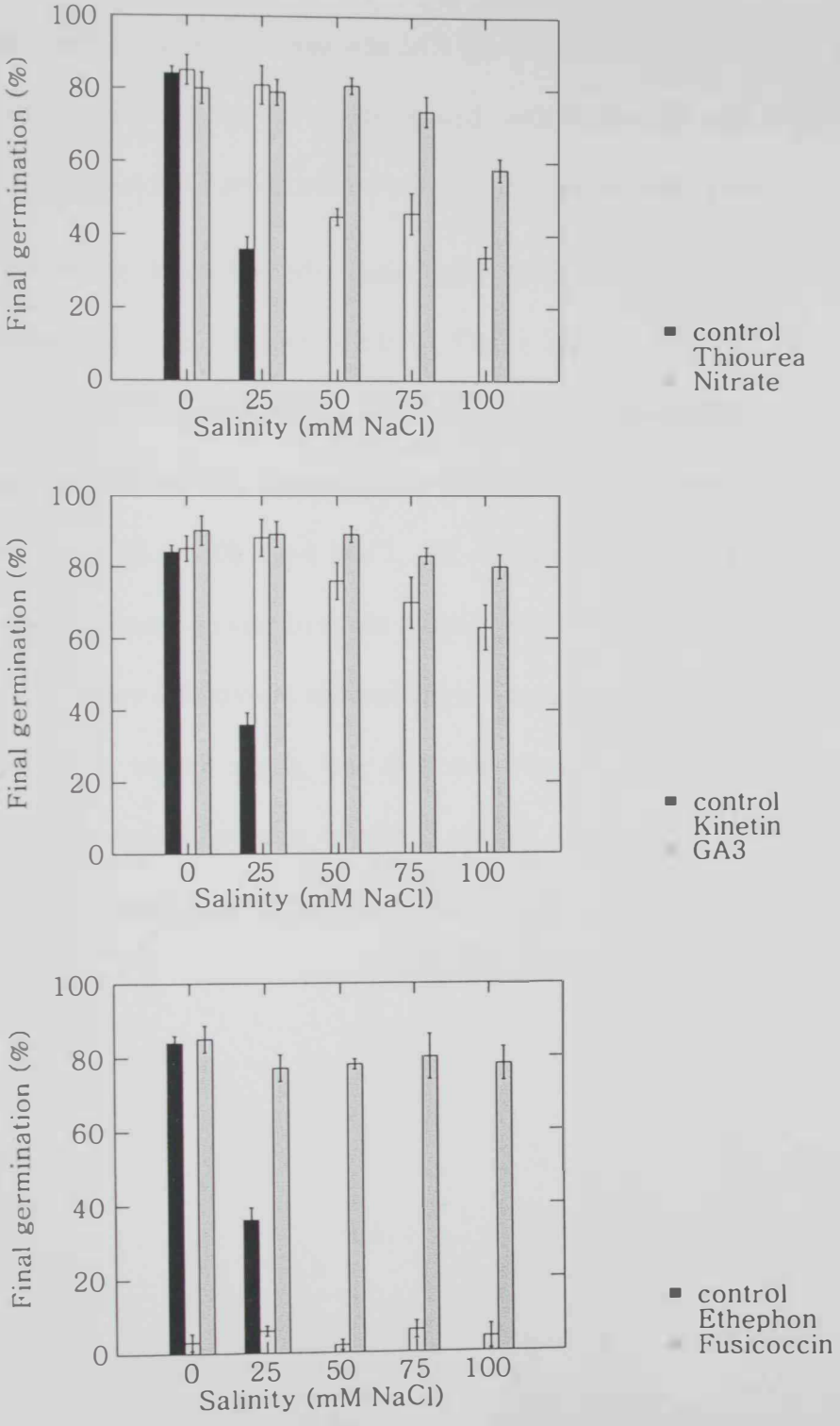
Table 15: Effects of dormancy regulating chemicals and NaCl concentration on final germination percentage (mean ± standard error) of *Cyprus conglomeratus* seeds

Salinity (mM NaCl)	Dormancy regulating chemicals						
	Control	**Fusicoccin**	**GA3**	**Kinetin**	**Nitrate**	**Thiourea**	**Overall**
0	84.0 ± 1.6	85.0^{+} ± 3.0	90.0^{+} ± 3.5	85.0^{+} ± 3.0	80.0^{+} ± 3.7	85.0^{+} ± 3.4	84.8 ± 1.3
25	36.0 ± 2.8	77.0*$^{+}$ ± 3.0	89.0*$^{+}$ ± 3.0	88.0*$^{+}$ ± 4.3	79.0*$^{+}$ ± 3.0	81.0*$^{+}$ ± 4.4	75.0 ± 3.9
50	0.0 ± 0.0	78.0*$^{+}$ ± 1.2	89.0*$^{+}$ ± 1.9	76.0*$^{+}$ ± 4.3	81.0*$^{+}$ ± 1.9	45. 0* ± 1.9	61.5 ± 6.5
75	0.0 ± 0.0	80. 0*$^{+}$ ± 5.2	83. 0*$^{+}$ ± 1.9	70.0* ± 6.0	74. 0*$^{+}$ ± 3.5	46.0* ± 4.8	58.3 ± 6.2
100	0.0 ±0.0	78. 0*$^{+}$ ± 3.8	80.0*$^{+}$ ± 2.8	63.0* ± 5.3	58.0* ± 2.6	34.0* ±2.6	52.2 ± 5.9
Overall	24 ± 7.6	79.6 ± 1.5	86.2 ± 1.4	76.4 ± 2.8	74.4 ± 2.3	58.2 ± 5.0	66.5 ± 2.5

Values with asterisk in the same salinity treatment significantly differ from the control of that treatment.

$^{+}$ not differ significantly from non-saline non-treated seeds.

Figure 13: Effects of dormancy regulating chemicals and NaCl concentration on final germination percentage (mean ± standard error) of *Cyprus conglomeratus* seeds



3.6.2 Effects on Germination Rate

The effect of DRC and salinity and their interaction on germination rate of *C. conglomeratus* seeds was significant ($P<0.001$, Table 16). Germination rate of non-saline treated seeds *C. conglomeratus* was 36.9 (the maximum value of the Timson index = 50). Overall germination rate of seeds treated with fusicoccin and GA3 was significantly greater than that it for seeds treated with kinetin, nitrate and thiourea.

For non-saline treated seeds, fusicoccin, GA3, nitrate and thiourea did not affect significantly germination rate (Table 17, Figure 14).

In the absence of DRC germination rate was significantly reduced in 25 mM NaCl (Timson index was 25), compared to that of non-saline treated seeds. No germination happened in 50 – 100 mM NaCl. All dormancy regulating substances significantly increased germination rate in seeds treated with 25 mM NaCl. In general fusicoccin and GA3 were more effective in enhancing germination rate in all salinities, compared to that of non-saline treated seeds, but this was clearer in lower than in higher salinities. However, germination rates of seeds treated with kinetin, nitrate and thiourea were significantly lower than that for non-saline treated seeds (Table 17, Figure 14)

Table 16: Two way ANOVA testing the effect of dormancy regulating chemicals and NaCl concentration on germination rate of *Cyprus conglomeratus* seeds

Source	Df	Mean-Square	F-ratio	P
DRC	5	15.087	3780.3	<0.001
Salinity	4	3.139	786.6	<0.001
DRC*Salinity	20	2.221	556.4	<0.001
Error	90	0.004		

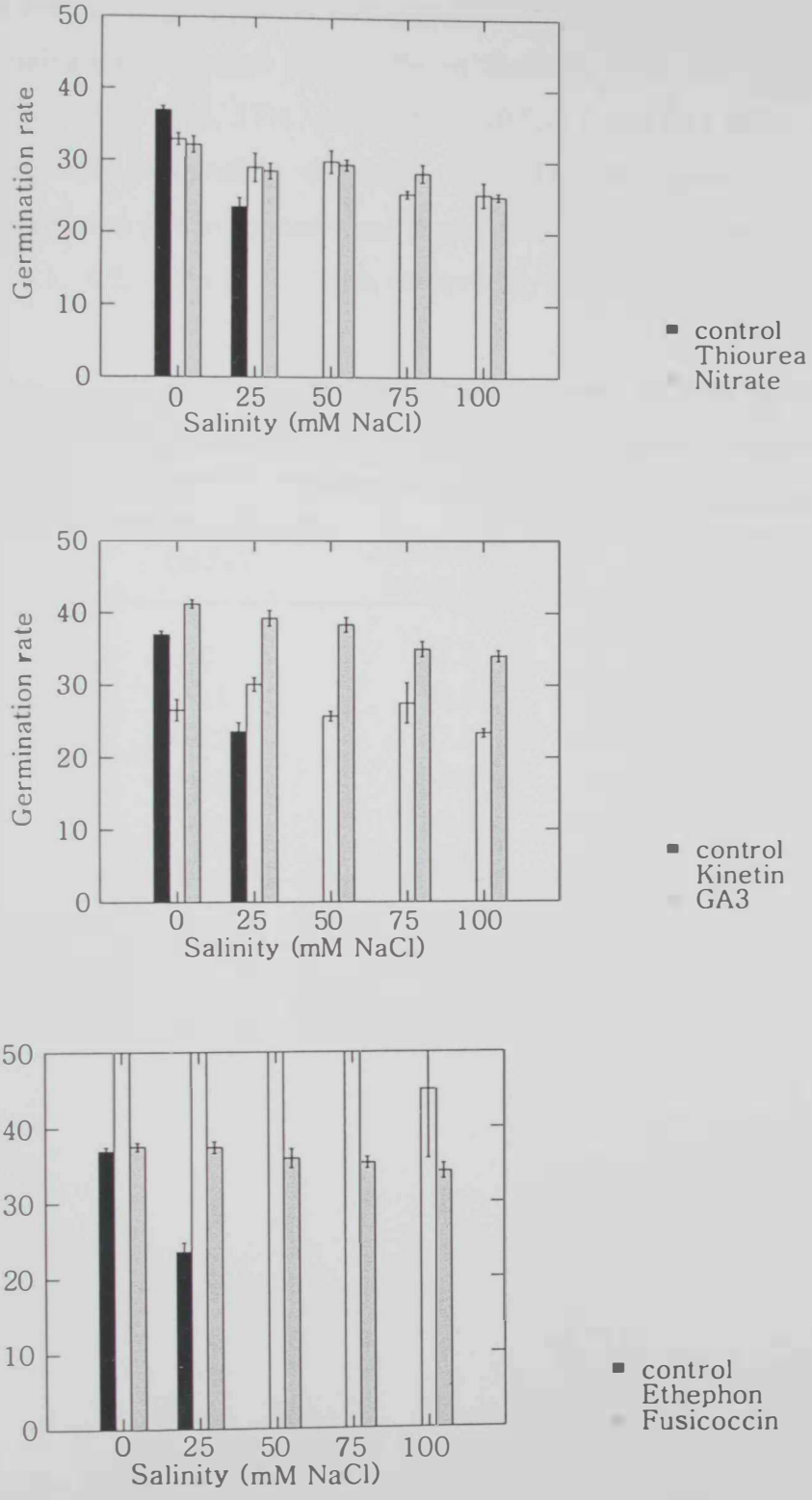
Table 17: Effects of dormancy regulating chemicals and NaCl concentration on germination rate (mean Timson index ± standard error) of *Cyprus conglomeratus* seeds

Salinity (mM NaCl)	Dormancy regulating chemicals						
	Control	**Fusicoccin**	**GA3**	**Kinetin**	**Nitrate**	**Thiourea**	**Overall**
0	36.9 ± 0.5	37.5^{+} ± 0.5	41.2^{+} ± 0.5	26.6* ± 1.2	32.2^{+} ± 1.0	32.9^{+} ± 0.7	34.6 ± 1.1
25	23.6 ± 1.0	37.4^{+}* ± 0.7	39.3^{+}* ± 0.9	30.1* ± 0.8	28.6* ± 0.9	29.1* ± 1.7	31.2 ± 1.2
50	0.0 ± 0.0	35.9^{+}* ± 1.1	38.4^{+}* ± 0.9	25.7* ± 0.6	29.5* ± 0.6	29.9* ± 1.3	26.6 ± 2.6
75	0.0 ± 0.0	35.3^{+}* ± 0.6	35.0^{+}* ± 0.9	27.5* ± 2.3	28.4* ± 1.0	25.5* ± 0.5	25.3 ± 2.5
100	0.0 ± 0.0	34.1^{+}* ± 0.9	34.0^{+}* ± 0.7	23.4* ± 0.5	25.1* ± 0.4	25.4* ± 1.4	23.7 ± 2.4
Overall	12.1 ± 3.5	36.0 ± 0.4	37.6 ± 0.7	26.7 ± 0.7	28.7 ± 0.6	28.6 ± 0.8	28.3 ± 1.0

Values with asterisk in the same salinity treatment significantly differ from the control of that treatment.

$^{+}$ not differ or significantly greater than non-saline non-treated seeds.

Figure 14: Effects of dormancy regulating chemicals and NaCl concentration on germination rate (mean Timson index ± standard error) of *Cyprus conglomeratus* seeds



3.7. Effect of Polyethylene osmotic pressure on final germination and germination rate of *Cyperus conglomeratus* seeds

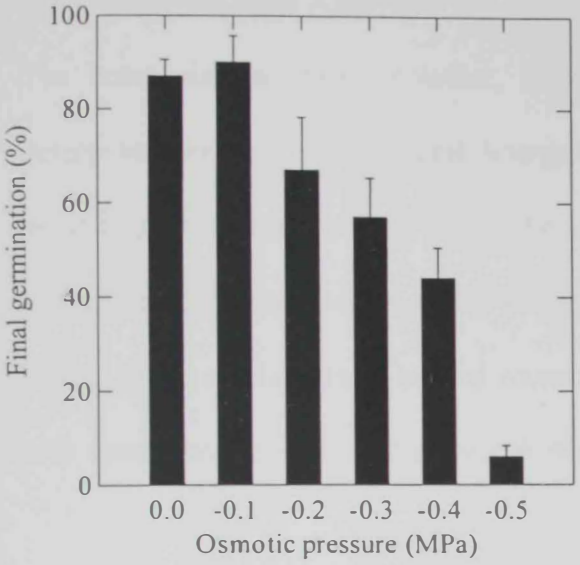
One-way ANOVA showed significant effects ($P<0.01$) for polyethylene osmotic pressure on both final germination and germination rate of *Cyperus conglomeratus* seeds. Final germination increased from 87% in distilled water to 90% in -0.1 MPa, but then decreased to 67%, 57% and 44% in -0.2,-0.3 and -0.4 MPa, respectively, and it was completely inhibited in -0.5 MPa (Table 18 and Figure 15(a)). In addition, Timson germination rate index decreased from 34.3 in distilled water to 32.5, 31.4, 27.9 and 20.8 in -0.1, -0.2, -0.3 and -0.5 MPa, respectively (Table 18 and Figure 15(b)).

Table 18: Effect of Polyethylene osmotic pressure on final germination and germination rate index (mean ± standard error) of *Cyperus conglomeratus* seeds

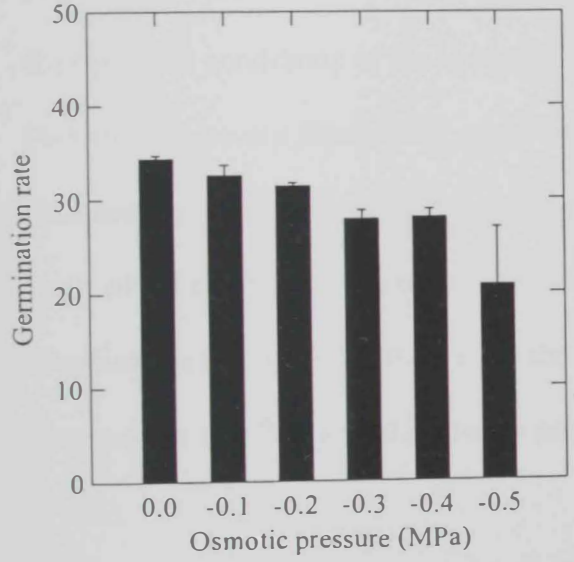
Osmotic pressure (MPa)	Final germination		Germination Rate index	
	Mean (%)	SE	Mean	SE
0	87.0	3.0	34.3	0.3
-0.1	90.0	4.7	32.6	0.9
-0.2	67.0	9.4	31.4	0.3
-0.3	57.0	7.0	27.9	0.8
-0.4	44.0	5.4	28.1	0.7
-0.5	6.0	2.0	20.8	4.6

Figure15 : Effect of Polyethylene osmotic pressure on final germination and germination rate index (mean ± standard error) of *Cyperus conglomeratus* seeds

(a) Final germination



(b) Germination rate



3.8 Composition and Quality of Seeds

3.8.1 Compositional Analysis:

The compositional data [moisture, ash, fat, protein, total carbohydrates, ADF (acid detergent fiber) and NDF (neutral detergent fiber)] of the samples collected from different areas is presented in Table 19. It can be seen from Table 19 that the seeds are rich in fat and protein. The high values of fat and protein in sample collected from Zakhir area could be due to the availability of treated water from a close by sewerage treatment plant as the area sometimes gets wet by the over flowing water from the treatment plant.

3.8.2. Supercritical Fluid Extraction of C. *conglomeratus* Oil:

A combination of two factors temperature and pressure of CO_2 was studied for obtaining the optimum conditions of the extraction yield. The CO_2 volume (200 mL) and flow rate (4-5 mL/min) were fixed in all runs. Table 20 provides data on the percent yield of oil obtained by supercritical fluid extraction (SFE) under different conditions. Maximum yield of 28.6% was obtained at the intermediate temperature and the highest pressure combination (50 °C and 400 bar). On the other hand, the combination between the highest temperature (60 °C) and the lowest pressure (200 bar) gave the least extraction yield (1.6%).

The effect of pressure on the extraction yield is shown in Figures 16-18 for different temperatures (40, 50 and 60 °C, respectively). At all temperatures, the extraction yield increased with pressure.

Table 19: Compositional analysis of Cyprus *conglomeratus* seeds*

Sampling Area	Moisture %	% on Dry weight basis					
		Ash	Fat	Protein (N x 6.25)	Total Carbohydrates	ADF	NDF
Al Ain	1.49	9.21	29.2	13.0	48.6	26.6	42.7
Dubai	3.29	6.97	29.2	13.5	50.3	21.0	36.1
Abu Dhabi	2.14	7.65	29.5	12.9	50.0	NA	NA
Zakhir	3.11	6.95	30.2	14.6	48.3	NA	NA
Average	**2.51**	**7.74**	**29.5**	**13.5**	**49.3**	**23.8**	**39.4**

*Average of duplicate determinations NA=Not analyzed

Table 20: Percent yield of C. *conglomeratus* oil under different SFE conditions

T (°C)	P (bar)	CO_2 density (g/mL)	Yield % (Mean ± SEM)
40	200	0.840	6.7 ± 1.28
40	300	0.910	16.8 ± 2.55
40	400	0.956	27.2 ± 0.45
50	200	0.784	3.3 ± 0.39
50	300	0.870	18.8 ± 1.13
50	400	0.923	28.6 ± 3.51
60	200	0.724	1.6 ± 0.11
60	300	0.830	19.7 ± 0.38
60	400	0.890	25.0 ± 0.95

Figure 16: Effect of pressure on the extraction yield of C. *conglomeratus* oil at 40 °C

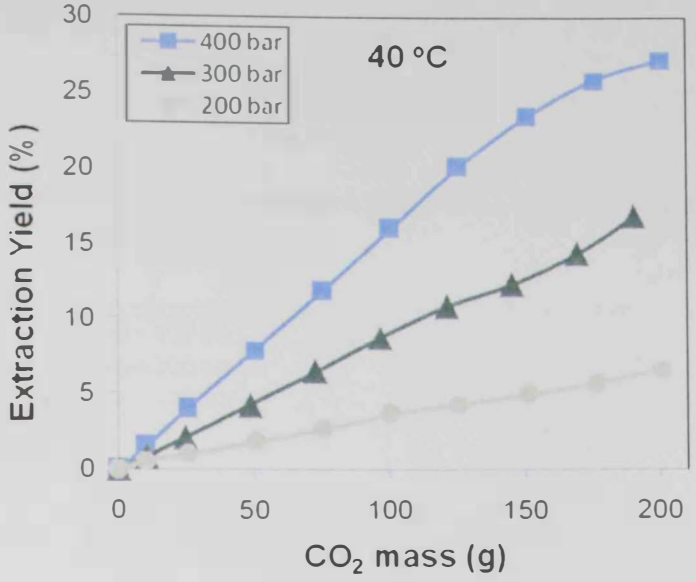


Figure 17: Effect of pressure on the extraction yield of C. *conglomeratus* oil at 50 °C

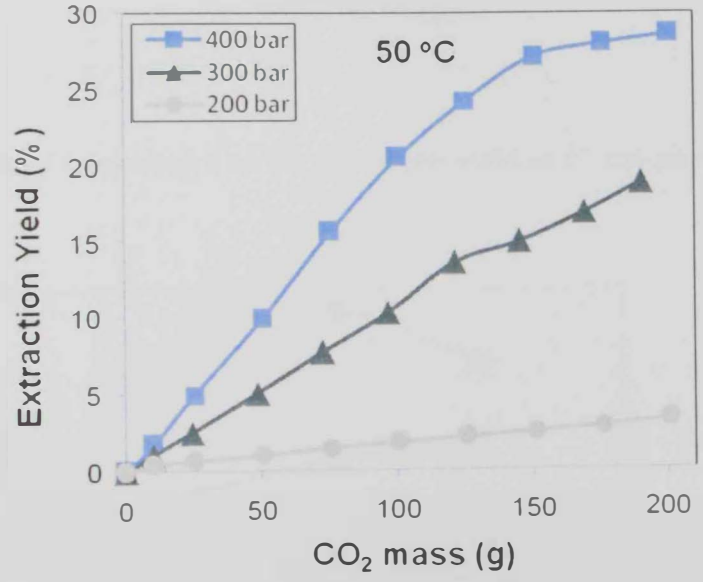


Figure 18: Effect of pressure on the extraction yield of C. *conglomeratus* oil at 60 °C

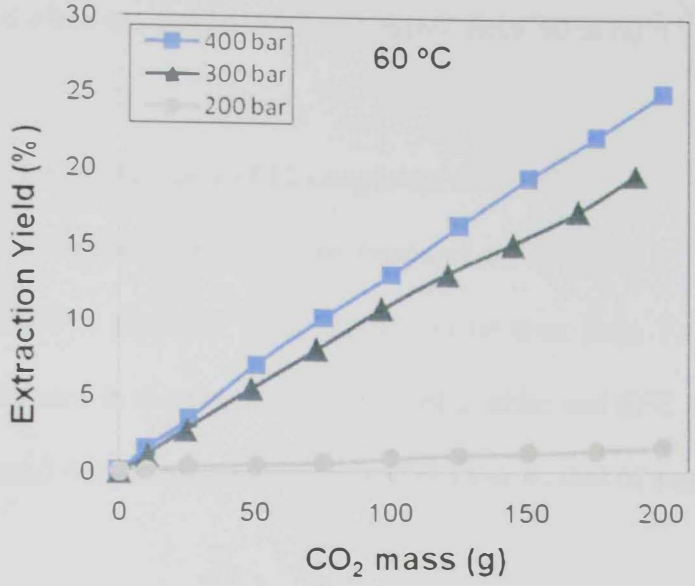
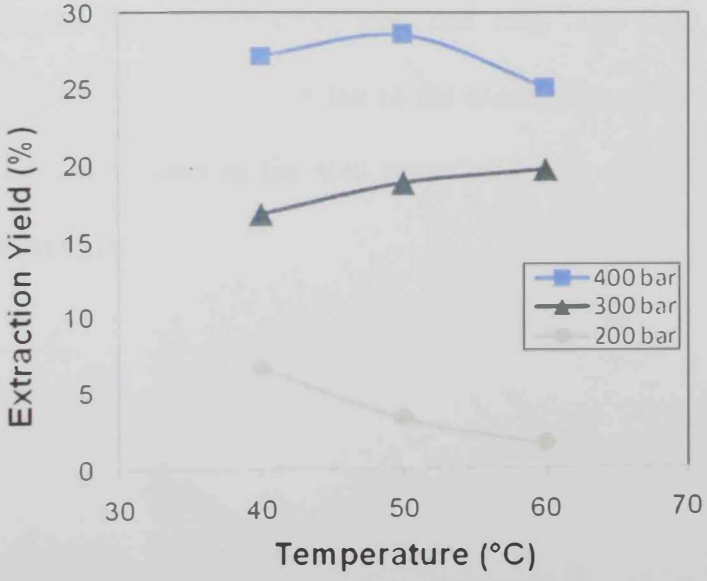


Figure 19: Effect of temperature on the extraction yield of C. *conglomeratus* oil



The effect of temperature on the extraction yield was different at different pressures (Figure 19). At 200 bar, extraction yield decreased when the temperature was increased from 40 to 60 °C. At 300 bar, the opposite behavior was observed; extraction yield increased with an increase in temperature. However, at the higher pressure (400 bar), extraction yield increased when the temperature was increased from 40 to 50 °C while the yield decreased when the temperature was raised from 50 to 60 °C.

3.8.3 Fatty Acid Composition of C. *conglomeratus* Oil

The fatty acid composition of *C. conglomeratus* oil obtained by both soxhlet and SFE extraction methods is given in Table 21. It can be seen from Table 21 that there is no significant difference in the fatty acid profile of soxhlet and SFE extracted oil. The oil is rich in unsaturated fatty acids and the oil is very close to that of sunflower oil.

3.8.4 Mineral Composition of C. conglomeratus Seeds

Table 22 provides data on the mineral composition of seeds collected from different areas. It can be seen from the table that seeds are rich in nutrient minerals especially potassium, magnesium, phosphorous, iron and zinc. The high values in the sample collected from Zakhir area could be due to the availability of treated water from a close by sewerage treatment plant as the area sometimes gets wet by the over flowing water from the treatment plant.

Table 21: Fatty acid composition of C. *conglomeratus* oil

Fatty Acid	Soxhlet Extracted oil	SFE Extracted oil
Caprylic acid (C8:0)	Traces	Traces
Capric acid (C10:0)	0.1	0.1
Lauric acid (C12:0)	0.1	0.1
Myristic aicd (C14:0)	0.16	0.15
Palmitic acid (C16:0)	7.47	7.61
Palmitoleic aicd (C16:1)	0.12	0.11
Steric acid (C18:0)	3.52	3.57
Oleic acid (C18:1)	73.8	73.2
Lenoleic acid (C18:2)	13.1	13.2
Lenolenic acid (C18:3)	0.56	0.58
Arachidic acid (C20:0)	0.12	0.13
Arachidonic acid (C20:1)	0.27	0.25
Behenic aicd (C22:0)	0.39	0.38
Erucic acid (C22:1)	0.17	0.18

Table 22: Mineral composition of C. *conglomeratus* seeds

Sampling Area	Mineral Mg/kg									
	Na	K	Ca	Mg	P	Fe	Cu	Zn	Pb	Cd
Al Ain	206	2829	828	2236	3064	189	8.69	50.8	0.18	0.11
Dubai	373	3262	1324	2434	3637	196	9.99	54.2	0.25	0.13
Abu Dhabi	357	3221	670	2173	3484	176	10.30	58.0	0.20	0.10
Zakhir	250	2802	761	2370	3778	204	9.31	50.3	0.30	0.21
Average	**297**	**3029**	**896**	**2303**	**3491**	**191**	**9.57**	**53.3**	**0.23**	**0.14**

CHAPTER 4

DISCUSSION

4. Discussion

4.1. Maternal Effects

The maternal environment during seed development and maturation has an important impact on the relative dormancy of the seed. For example, both high temperatures and lower levels of soil moisture decreased seed dormancy in *Avena fatua* (Sawhney and Naylor, 1982). In the present study, seeds of *C. conglomeratus* produced on dry sand dunes (AlWathbah, Al Khattem and Dubai) produced higher germination percentages whereas those produced in the two sites of Al-Ain (Manaseer and Industrial area) produced significantly lower germination. Plants growing on sand dunes of AlWathbah, Al Khattem and Dubai are exposing to water stress because of the high infiltration rate of the coarse sands of these dunes. Premature drying has been found to reduce the period of seed dormancy in wheat, and this effect was correlated with a reduction in abscisic acid (a germination inhibitor) content of developing kernels (Sawhney and Naylor, 1982). Benech Arnold *et al.* (1992) determined that water stress during seed development reduced dormancy of *Sorghum halepense* seeds through modifications in the properties of the glumes that, apparently, result in an enhancement of their permeability to oxygen diffusion. On the other hand, *C. conglomeratus* plants of the Manaseer site are grown with ornamental plants and hence receive extra-water through irrigation. Similarly, the population in the industrial site of Al-Ain is located on sand dunes nearby a site in which sewage treated water is dumped. Several studies documented that water addition in the maternal environment cause a significant decrease in germination percentage and rate of several species, such as *Sinapis arvensis* (Wright *et al.*, 1999; Luzuriaga *et al.*, 2006), *Malva parviflora* (Michael *et al.*, 2006). Wright *et al.* (1999) proposed that adequate moisture during seed formation is expected to result in the production of more dormant seeds than in drier conditions, probably because better developed seeds are produced. The

lowest germination for *C. conglomeratus* was recorded for seeds of Liwa population. During the study year (2008), the sand dunes of Madinat Zayed (nearest station to Liwa) received only 8 mm rainfall, compared to about 60 mm in Abu Dhabi and 136 mm Dubai (Table 1). Consequently, the lower germination of seeds of Liwa might be attributed to the extremely water stress which would lead to improper seed filling and high rate of seed abortion.

The most important factors that affect maternal plants have been suggested to be temperature, rainfall and day length (Baskin and Baskin, 1998). Generally, seeds produced by plants at higher temperatures, water stress and shorter days have higher germination percentage and/or rates (i.e., lower dormancy) than those produced at lower temperatures, long days and favourable moisture conditions. The lower germination of seeds from the harshest Liwa site would not be explained by the higher water stress resulted from the extremely lower rainfalls in that area. Rather, it would be explained in the light of the improper seed filling and high rate of seed abortion, as mentioned above. Environmental conditions under which plants are grown can affect seed germination by affecting their chemical composition and seed provisioning (e.g., mineral, photosynthetic and phytohormone resources) throughout the growing season (Roach and Wulff 1987; Baskin and Baskin 1998; Galloway 2002). In addition, maternal environment can change the structure and thickness of the seed coat (Lacey *et al.* 1997; Luzuriaga *et al.* 2006).

4.2. Innate Dormancy in *Cyprus conglomeratus* seeds

Seed dormancy is the failure or block of a viable seed to complete germination under physical conditions that normally favor the process (Hilhorst, 1995; Koornneef *et al.*, 2002). It could be coat-imposed and/or determined by the embryo itself and is a temporary. Germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expanse growth in some species, such as *Pisum sativum*

(Petruzzelli *et al.*, 2000). In *C. conglomeratus*, the testa (seed coat) has no hindrance during the germination, as most of the seeds imbibe water after seed soaking. This might explain the lower seed dormancy of fresh harvested seeds of *C. conglomeratus*.

Seed dormancy and germination are complex developmental processes that are regulated by a variety of endogenous and environmental signals. Plant growth regulators such as gibberellic acid (GA3), abscisic acid (ABA), kinetin and ethylene are known to influence the dormancy status of seeds (Karssen 1995). According to the revised hormone-balance hypothesis for seed dormancy (Karssen and Laçka, 1986), ABA and GA act at different times and sites during "seed life". ABA induces dormancy during maturation and GA3 plays a key role in the promotion of germination (Leubner-Metzger 2003). There is considerable evidence that ABA is an important positive regulator of both the induction of dormancy and the maintenance of the dormant state in imbibed seeds following shedding. While dormancy maintenance also depends on high ABA:GA ratios, dormancy release involves a net shift to increased GA biosynthesis and ABA degradation resulting in low ABA:GA ratios (e.g. Ali-Rachedi et al., 2004; Cadman et al., 2006, Finch-Savage and Leubner-Metzger 2006). In the present study, however, GA3 had no effect in enhancing germination in non-saline treated seeds.

The impact of different dormancy regulating chemicals on innate dormancy has been studied in several species of subtropical deserts in both Pakistan and the UAE. In subtropical halophytes of Pakistan, dormancy regulating chemicals had either no effect or a negative effect on innate dormancy on some species, such as *Aeluropus lagopoides, Halopyrum mucronatum, Limonium stocksii, Salsola imbricata, Sporobolous ioclados*, and *Urochondra setulosa* (see references in Khan and Gul, 2006). In the invasive *Prosopis juliflora* in the UAE, innate dormancy was not improved by GA3, kinetin, thiourea or fusicoccin (El-Keblawy *et al.* 2005). Similarly, only thiourea, out of six

chemicals, succeeded to improve germination of non-saline treated seeds of both *L. scindicus* and *P. turgidum* and the other five chemicals had no or negative impact on the germination (El-Keblawy *et al.* 2009). The present study showed that seeds of *C. conglomeratus* have little innate dormancy; 84% of the seeds germinated in distilled water. In addition, none of the studied chemicals improved the germination of the non-saline treated seeds.

4.3. Impacts of light and Temperature

It has been reported that seeds of many species require light or germinate better in light than in dark. In a glasshouse experiment, 15 out of 17 herbaceous species from highlands of Ethiopia, Teketay (1998) showed that percent germination for seeds incubated in daylight was significantly higher than those incubated in darkness or under leaf shade indicating the induction of secondary dormancy during incubation in either darkness or leaf shade. In addition, the germination tests for 32 species of sedges after cold stratification indicated that the germination in light was considerably higher in all but two species compared to that in darkness (Schutz and Rave 1999). Furthermore, in four species from Alaska, cold stratified seeds germinated greater in longer than in shorter day length (Densmore 1997). In two sedge species (*Juncus acutus,* Juncaceae) and *Schoenus nigricans,* Cyperaceae), total darkness delayed germination in relation to the 12 h photoperiod. Seeds of the two species did not germinate at 30 °C in darkness, but they did at the same temperature with 12 h (Martinez-Sanchez et al. (2006). The results of the present are in line with these finding: *C. conglomeratus* seeds germinated significantly greater in light, compared to the darkness.

The ecological conditions prevailing in a given habitat will affect water imbibition and subsequent stages of seed germination. The environmental factors affecting germination under natural conditions depend on the nature of the soil, its chemical

composition and its physico-chemical structure and the seed's depth in the soil. In the present study, both light and temperature and their interaction have significant effect on final germination of *C. conglomeratus* seeds. Generally, the final germination was significantly greater at fluctuating, compared to it at constant temperatures. In addition, light germination was greater than dark germination at all fluctuating temperatures, but the difference was significant only at the higher temperatures (25/35 and 30/40 oC). Soil depth influences the light and the daily and yearly fluctuations in temperature (Pons 1992). Light intensity and temperatures are higher at soil surface than at lower depths (Mayer and Poljakoff-Mayber 1989).

The fact that light enhances germination in several species indicates that the burial of seeds caused by the movement of sand or fauna activity may reduce germination of these species. Other factors, such as low temperature fluctuations in deeper soil horizons, may also prevent the germination of buried seeds (Schutz and Milberg, 1997). In the present study, the light requirement for germination of *C. conglomerates* seeds at higher temperatures (25/35 and 30/40 oC) ensures that they will germinate successfully on or near the soil surface when other conditions are suitable for seedling emergence. Such result might explain the high seedling emergence observed in disturbed sand dunes, especially after effective rainfall at the end of the rainy season (March –April, El-Keblawy *et al.*, 2009).

The present study showed that germination of *C. conglomeratus* was higher at higher fluctuating temperatures (30/40 °C), compared to lower fluctuating and constant temperatures. Similar result has been reported in two salt marsh grasses (*Aeluropus lagopoides* and *Sporobolus madraspatanus*) as a noticeable increase in germination percentage was recorded at fluctuating temperatures, as compared to it at constant temperatures. In many cases, the increase was greater than 50% over the germination at

constant temperature (Joshi *et al.*, 2004). The greater germination at higher fluctuating temperatures (30/40 °C) has an important ecological significance because similar fluctuations in day and night temperatures during seed germination in nature might be promoting the germination process. This agrees with the late emergence of *C. conglomeratus* seedling at the end of the rainy season, when the fluctuations at surface temperature is high.

Fluctuating temperatures could be an important environmental signal for seeds located deep in the soil or buried in the mud under water (Ekstam *et al.* 1999). Thompson et al. (1977) reported that the requirements for diurnal fluctuations in temperature are characteristic of the germination of species from particular types of habitat and provide mechanisms which cause seeds to germinate at times and in places favourable for seedling establishment. Rojas-Arechigo and Vazquez-Yanes (2000) reviewed the response of cactus seed germination to temperature incubation of more than 50 species in 20 studies and concluded that alternating temperatures stimulate germination than constant temperatures. Out of 16 species from the highlands of Ethiopia, 11 showed significantly greater germination in alternating compared to constant temperatures. Alternating temperatures is also required for germination of many other species including *Setaria faberi,* and *Panicum dichotomiflorum* (Fausey and Renner 1997), *Poa pratensis* (Aamlid and Arntsen 1998), *Medicago sativa* (Hall *et al.* 1998), *Eriochloa* villosa (Bello *et al.* 1998), *Persicaria hydropiper, P. lapathifolia,* and *P. longiseta* (Araki and Washitani 2000). In addition, Schutz (1999) has categorized 34 sedges species into two groups: species requiring constant temperature for considerable germination, and other responded only to strong fluctuation in temperatures to attain higher germination. Sedges occurring in dry habitats showed a lower sensitivity to alternating temperatures than sedges of wet habitats. In *C. conglomeratus*, which is sedge of the hyperarid habitats of the UAE desert

require fluctuating high temperatures for optimum germination. Little or no germination occurred at the constant temperatures. Species requiring fluctuating temperatures usually germinate at soil surface and consequently require disturbances, which result in the formation of gaps and/or expose seeds to soil surface if they are buried deep into the soil or dispersed under vegetation canopy (Teketay 1998). The requirement of higher alternating temperatures for the germination of *C. conglomeratus* seeds explains the capacity of this species to colonize disturbed sandy habitats, especially when rainfall happen at the end of the growing season (April – May). It has been reported that this species can benefit from any disturbance and has the capacity to colonize sand habitats before any other species following disturbance and rain events (Ferguson et al., 1998). On the other hand, lower germination in both light and dark at lower temperature (20/30 °C) might explain the lower seedling emergence in cooler months of the season (Dec. – Feb), even after effective rainfall.

4.4. Salinity effects

Seeds of *C. conglomeratus* showed little dormancy when germinated in distilled water (non-saline control). The germination was greatly reduced in 25 mM NaCl and completely inhibited in 50 mM NaCl. The monocotyledonous plants are sensitive to salinity during the germination stage, but *C. conglomeratus* is even more sensitive. The lowest NaCl concentrations tolerated by monocotyledonous halophytes was 320 mM NaCl for *Halopyrum mucronatum* (Ungar, 1974), 300 mM NaCl for *Puccinellia distans* (Harivandi *et al.*, 1982), 200 mM NaCl for *Panicum coloratum* (Perez et al., 1998) and *P. hemitimon* (Hester *et al.*, 1998) and 200 mM NaCl for *Hordeum jubatum* (Khan & Ungar, 2001b). In the UAE deserts, El-Keblawy assessed the salinity tolerance in five grasses and found them very sensitive to salinity during germination. Low proportions of

the seeds of *Dichanthium annulatum* (5%), *Cenchrus ciliaris* (12%) and *Pennisetum divisum* (20%) were germinated in 100 mM NaCl (El-Keblawy 2006). In addition, Seeds of *Lasiurus scindicus* and *Sporobolus arabicus* germinated to 10 and 5%, respectively, in 200 mM NaCl (El-Keblawy 2006). Complete inhibition, or noticeably poor seed germination even in low concentrations of salts, clearly indicate that seeds of *C. conglomeratus* are extremely salt sensitive. This would explain its ecological behaviour that is showing great abundance in the sandy habitats that are characterized by very low salinity. The result also explains the rare occurrence of *C. conglomeratus* in salty affected sites.

Salt tolerance at germination seems to have no relation to the tolerance level during seedlings growth (Johnson, 1991). It has been reported that grasses like *Panicum coloratum* (Perez et al., 1998) and *P. hemitimon* (Hester et al., 1998) germinate well in NaCl concentrations up to 200 mM, but seedling growth is significantly retarded, showing a lethal effect under these salt concentrations. Similarly, Ungar (1996) observed that seeds of *Atriplex patula* were less affected by salinity than the growing plants. *Cyperus conglomeratus* is one of the most sensitive plants to salinity. It is usually growing on sand dunes, which are characterized by very low in soil nutrients (Western, 1989). The present study indicated that germination stage of *C. conglomeratus* is as sensitive as, or even more, than vegetation stage.

Germination failure in saline soils has been attributed to both osmotic and toxic effects. The osmotic effects is usually attributed to declining soil solute potential and the toxicity effects is due to uptake and/or accumulation of some ions, as sodium and chloride, in the seeds (Poljakoff-Mayber *et al.* 1994; Khan *et al.,* 2001a; Tobe *et al.,* 2001). The toxicity effect of salinity compromise enzyme function and disrupt metabolic processes (Baldwin *et al.,* 1996). Consequently, toxic effect of salinity is exaggerated by

high temperatures in many species such as *Salsola imbricata* (El-Keblawy et al. 2007), *Haloxylon recurvum* (Khan and Ungar, 1996), and some *Atriplex* species (Potter *et al.*, 1986). The greater detrimental effect of high salinities at higher temperatures indicates that toxicity of Na^+ usually causes irreversible damage (Bewley and Black, 1994; Khan and Ungar, 1996). In the present study, however, the greatest salinity tolerance for *C. conglomeratus* seeds was observed at that the highest temperature (30/40 °C). In addition, recovery germination of ungerminated seeds in saline solutions was greatest at the 30/40 °C. The higher oil contents (30% of the dry weight) in the seeds of *C. conglomeratus* might have a role in the greater salinity tolerance at higher temperatures. Further physiological studies are required to explain the unusual great ability of the seeds to tolerate salinity at the high temperature.

Light and salinity interact during germination in a number of plants. In some halophytic shrubs and forbs, germination inhibition in saline solution was more substantial in dark than in light (Khan & Ungar, 1997). For example, the increase in NaCl concentration progressively inhibited seed germination in *Allenrolfea occidentalis*, and this inhibition was greater in the dark than in light (Gul and Weber, 1999). Similarly, germination was inhibited in four desert shrubs and a forb, following an increase in salinity, and this inhibition was more substantial in dark than in light (Khan and Ungar, 1997b). Also, both darkness and high salinity inhibited germination of *Limonium stocksii* (Zia and Khan, 2002). Furthermore, germination inhibition was greater for seeds of *Prosopis juliflora* in darkness, than in light (El-Keblawy and Al-Rawai 2005). In our study, however, the germination of *C. conglomeratus* was completely inhibited in higher salinities (75-100 mM NaCl) when seeds germinated in light, but attain about 20% when seeds germinated in darkness, especially at higher temperatures (25/30 and 35/40 °C). A similar result has been reported for halophytic *Sarcocornia fruticosa*. Germination in

darkness was higher than in light/darkness conditions at 4% of salinity, while an opposite trend was found in distilled water (Redondo *et al.* 2004). The dark requirement under saline conditions in *S. fruticosa* has been attributed to seed burial in soil fissures and dense vegetation that could reach to 100% in the habitat of this species (Redondo *et al.* 2004). The high temperature and darkness requirements for the germination of *C. conglomeratus* under saline conditions indicate that seeds buried in low-saline soils (75-100 mM NaCl) could germinate in summer after monsoon rainfalls. Sand drifts bury seeds in the low laying lands intervening sand dunes. Such lands receive leached nutrients from the surrounding sand dunes and usually are relatively more saline.

4.5. Germination Recovery

Halophyte seeds, unlike glycophyte seeds, can remain viable for long periods under extremely high salinity and germinate at a later time when the osmotic potential of the medium is raised (Uhvits, 1946; Boorman, 1968; Macke and Ungar, 1971; Ungar, 1978, 1995; Naidoo and Naicker, 1992). Recovery germination from higher salinities was very low in some species (e.g., *Zygophyllum simplex*, Khan and Ungar, 1997; *Halogeton* glomeratus, Khan *et al.*, 2001a; *Sarcobatus vermiculatus* (Khan et al., 2001b), *Sporobolus ioclades*, Khan and Gulzar, 2003). However, high salinity did not permanently injure seeds and germination fully recovered when seeds were transferred to distilled water in several other halophytes, including *Atriplex patula* (Ungar, 1996), *Suaeda fruticosa* (Khan and Unger, 1997), *Arthrocnemum macrostachyum, Sarcocornia fruticosa* and *Salicornia ramoissim* (Pujol *et al.*, 2000) *Salicornia rubra* (Khan *et al.*, 2000). In the present study a significant proportion (differs depending the temperature of incubation) of *C. conglomeratus* seeds recovered their germination when transferred from 100 mM NaCl to distilled water. A similar result had been reported for the glycophyte *Schinopsis*

quebracho-colorado in the arid subtropical biome of the South America (Meloni et al. 2008). In addition, seeds of the annual glycophyte *Diplotaxis harra* recovered germination when treated with 150 mM NaCl, but not after treatment with 200 mM NaCl (Tlig et al. 2008)

The variation in germination recovery responses has been attributed to the temperature regime to which they are exposed. Greater ability to recover germination has been reported at cooler temperatures for some species (e.g., *Suaeda fruticosa*, Khan and Ungar, 1997; *Salsola imbricata*, El-Keblawy *et al.*, 2007), and *Haloxylon salicornicum* (El-Keblawy and Al-Shamsi 2008), but at warmer temperatures in other species (e.g., *Aeluropus lagopoides*, Gulzar and Khan, 2001; *Halogeton glomeratus*, Khan *et al.*, 2001). Our results showed that the recovery germination of *C. conglomeratus* was higher at 30/40 °C, compared to at other lower temperatures. The greater recovery at higher temperature indicate the ability of seeds of *C. conglomeratus* to germinate after monsoon rainfalls that are taking place during some summers. However, emerged seedlings at that time would be at greater risk as the soil is rapidly drying at the high temperatures because of high evaporative rate. The presence of shallow roots in *C. conglomeratus* with a sandy sheath around them enables the plants to absorb fog and dew precipitations. In addition, the rapid recovery after transferring *C. conglomeratus* seeds to distilled (El-Keblawy, unpublished data) would ensure that ungerminated seeds exposed to saline conditions could germinate during periods of monsoon precipitation when stress is temporarily alleviated.

4.6. Impacts on Germination Rate

Seeds of most Mediterranean and desert species have dormancy characteristics or structural properties that prevent immediate germination of at least a proportion of the seeds (Gutterman, 1994; Bell *et al.*, 1995). In addition, these species might delay the

germination until their habitats receive enough rainfall that might secure seedling establishment later in the season (El-Keblawy 2003). Leaching of some germination inhibition from seed coat or structures surrounding the seeds might trigger germination when soils receive enough rainfall. In the present study, germination rate of *C. conglomeratus* was very slow; maximum germination rate index in non-saline treated seeds was about 25, out of 50, which is the maximum possible rate. This indicates that strategy in *C. conglomeratus* is to delay germination until soils receive enough water that would secure seedling establishment. The lower germination rate would be ecologically disadvantage as it would produce seedlings late in the season with inferior interspecific competition. Seedlings would occupy the space after other species with faster germination. *C. conglomeratus*, however, are mainly growing on sand dunes; where there are few species can compete with them. Few species can tolerate the instability of sand dunes and the low nutrient content of its soil.

Osmotic seed priming (osmopriming) is used to increase germination efficiency, especially speed of germination. Under this treatment, seeds are partially hydrated and begin the processes of germination, but emergence does not occur, and the seeds are subsequently dried. Priming has several effects on the physiology of seeds, including developmental advancement, decreased membrane permeability, dormancy breaking, and the induction of DNA and protein synthesis (Hudson et al. 2007). In our study, osmopriming of *C. conglomeratus* seeds resulted in germination enhancement; up to 35% of the seed germinated within few days when the seeds were transferred to distilled water at 30/40 °C. A similar rapid response to decrease in soil osmotic potential after seeds were exposed to low water potentials has been reported in many halophytic and gycophytic plants (Katembe et al. 1998; Gul and Weber, 1999; Zia and Khan, 2004; El-Keblawy et al . 2007; El-Keblawy and Al Shamsy 2008). For example, osmotic pre-

treatment significantly stimulated germination recovery of seeds of two *Atriplex* species (*Chenopodiaceae*), and their germination was 90% 2 d after transfer to distilled water (Katembe et al., 1998). Such rapid response is an important adaptation, since it would ensure that ungerminated seeds exposed to saline conditions could germinate during periods of precipitation when stress was temporarily alleviated. The enhanced germination speed for *C. conglomeratus* seeds recovered their germination after effective monsoon rainfalls might enhance the establishments of the emerged seedlings before soil drying.

4.7. Effect of Seed Storage

Many species produce seeds that do not germinate shortly after dispersal and require a period of species-specific afterripening through dry storage (Bewley and Black, 1982; Simpson, 1990; Baskin and Baskin, 1998). The parameters that determine seed after-ripening are moisture and oil contents, seed-covering structures, and temperature (Manz *et al.*, 2005 and references therein). Both storage conditions and duration are important factors in regulating the after-ripening process (Paterson et al., 1976; Peishi et al., 1999; Murdoch and Ellis, 2000). For example, in three Australian everlasting daisy species, short term storage (<18 months) at cool temperatures increased seed moisture content, reduced viability and did not promote germination, but storage at high temperatures decreased seed moisture content, maintained viability and improved germination (Peishi et al., 1999). In the present study, final germination of fresh seeds of *C. conglomeratus* (34.6%) was significantly greater, compared to it for seeds stored for 15 months (28.7%). This result indicates that storage either induced a secondary dormancy or resulted in viability loss of the seeds. After-ripening is prevented in very dry seeds; it requires seed moisture contents above a threshold value. This threshold moisture content is species-specific and lower in oilseeds compared with starchy seeds because they contain less

bound water when equilibrated at any given relative humidity (Qaderi 2005). It has been documented that seeds of *C. conglomeratus* contain about 30% of their dry weight oil that might greatly restrict the amount of available water during storage. At lower moisture contents, seeds may simply fail to after-ripen whereas at the higher moisture levels they may enter secondary dormancy or lose viability

4.8. Effects of Dormancy Regulating Substances

Change in growth regulators balance induced by salt stress may be a mechanism that induces dormancy in seeds (Ungar 1978). The role of various germination regulating chemicals such as proline, betaine, gibberellin, kinetin, nitrate, thiourea and ethephon in reducing the inhibitory effects of salinity on germination has been reported for several halophytes (Kabar 1987; Bewely and Black 1994; Plyler and Proseus 1996; Gul and Weber 1998; Khan and Ungar 1997, 2000, 2001 a,b,c, Khan et al. 2002, El-Keblawy et al. 2005). For example, in *Triglochin maritime*, the effect of low salinity on germination was alleviated by fusicoccin, kinetin, nitrate and thiourea, whereas, the reduction in germination at high salinity was partially countered by ethephon, kinetin, thiourea and nitrate (Khan and Ungar 2001). Similarly, salinity-enforced dormancy in *Sporobolus arabicus* seeds was not alleviated by kinetin, GA_3, and ethephon, but fusicoccin, nitrate, and thiourea significantly alleviated it (Khan and Ungar 2001). In addition, ethephon, fusicoccin, GA3, kinetin, thiourea, and nitrate promoted germination under low saline conditions in *Salicornia rubra*. At higher salinity, fusicoccin had no effect, whereas GA3, kinetin, and ethephon substantially alleviated salinity effects (Khan et al. 2002). The role of dormancy regulating substances on alleviating salinity induced dormancy has been also documented for some desert glycophytes. For example, the salinity-induced germination inhibition in *Panicum turgidum* was completely alleviated by exogenous application of

gibberellic acid and partially alleviated by the application of fusicoccin, kinetin and thiourea. Similarly, the germination inhibition of *Lasiurus scindicus* seeds in saline solution was completely alleviated by fusicoccin, gibberellic acid, nitrate and thiourea, but partially alleviated by kinetin (El-Keblawy et al 2009b). In other five glycophytic grasses of the UAE deserts, different germination regulating chemicals had little or no effect on alleviating germination inhibition in *Centropodia forsskalii* and *Pennisetum divisum*, but significantly alleviated it in *Tragus racemosus*, *Sporobolus spicatus* and *Eragrostis barrelieri*; so, the response of the different species differed for the different chemicals. Partial alleviation was observed in 100 mM NaCl by fusicoccin in *C. brevifolium*, by GA and Kinetin in *P. divisum* and by GA, kinetin and thiourea in *C. forsskalii*. GA was the most effective in alleviating the germination inhibition *E. barrelieri*. Nitrate was the most effective in *S. spicatus*, followed by fusicoccin and GA (El-Keblawy 2008). In the present study, fusicoccin, GA3 and kinetin, completely alleviated salinity induced dormancy of *C. conglomeratus* seeds in all salinity levels. Nitrate and thiourea completely alleviated the germination inhibition in lower salinities, but partially alleviated it in 100 mM NaCl.

In saline environments, high salt levels are known to impair seed germination of halophyte species (Debez et al. 2004). Despite the mechanisms of this inhibition remain unclear, such an inhibition may partly ascribed to the strong decline in gibberellic acid (GA3) levels during seed imbibition (Kabar and Baltelpe 1989). Interestingly, the exogenous application of GA3 is known to efficiently alleviate the harmful effect of salinity on halophyte germination (Debez et al. 2001; Khan et al. 1998). In addition,The exogenous application of GA3 was effective in mitigating NaCl salinity effect on germination of several halophytes, such as *Zygophyllum simplex* (Khan and Ungar, 2002), *Arthrocnemum indicum* L. (Khan et al 1998), and *Prosopis juliflora* (El-Keblawy et al.

2005). In the present study, GA3 significantly alleviated salinity induced dormancy in all salinity levels. Despite salinity significantly reduced germination in 25 mM NaCl and completely inhibited it in 50 mM NaCl, seeds of the two salinities did not differ from those germinated in distilled water when treated with GA3. This could be explained by the fact that GA3 may reduce the ABA level in seeds through the activation of their catabolism enzymes or by blocking the biosynthesis pathway (Toyomasu et al. 1994). Two functions for GA during seed germination have been proposed (reviewed in Kucera *et al.*, 2005). First, GA increases the growth potential of the embryo and promotes germination. Secondly, GA is necessary to overcome the mechanical restraint conferred by the seed-covering layers by weakening of the tissues surrounding the (Bewley and Black, 1994, Kucera *et al.*, 2005).

In addition to GA_3, kinetin, another growth regulator, completely alleviated the germination inhibition of *C. conglomeratus* seeds at higher salinities (100 mM NaCl). Both these growth regulators are known to alleviate the salinity-induced germination inhibition in *Brassica campestris* (Ozturk et al. 1993), *Halopyrum mucronatum* (Khan and Ungar 2001a), *Atriplex halimus* (Debez et al. 2001), *Atriplex triangularis*, *Atriplex stocksii* (Khan and Ungar, 2000), *Salicornia rubra* (Khan et al. 2002), *Ceratoides lanata* (Khan et al. 2004), *Prosopis juliflora* (El-Keblawy et al. 2005). The effect of kinetin on alleviation of salinity-induced dormancy partial in *Triglochin maritima* (Khan and Ungar 2001b), *Zygophyllum simplex* (Khan and Ungar 1997), but did not present in *Salicornia pacifica* (Khan and Weber 1986), *Zygophyllum qatarense* (Ismail 1990), *Sporobolus arabicus* (Khan and Ungar 2001c). Growth regulators, like GA3 and kinetin, induced a great increase in germination of chickpea (*Cicer arietinum*) seeds under salt stress, by increasing amylase activity in the cotyledons (Kaur et al. 1998). The increase in seed germination under salt-stressed, after exogenous application of GA_3 and kinetin, was

attributed to the ability of the growth regulators to reduce the moisture requirement or to enhance water uptake during germination and early seedling growth (Sastry and Shekhawat 2001).

Germination inhibition at higher salinity in *C. conglomeratus* was also completely alleviated by fusicoccin. The impact of fusicoccin on germination inhibition was complete in *Sporobolus arabicus* (Khan and Ungar 2001c), *Zygophyllum qatarense* (Ismail 1990), *Arthrocnemum macrostachyum*, but partial on *Triglochin maritima* (Khan and Ungar 2001b), but not present in *Salicornia rubra* (Khan et al. 2002). The alleviation effect of fusicoccin may be due to the stimulation of ATPase production, which increases during the early phases of germination to facilitate proton extrusion and K+ uptake (Stout 1988). Fusicoccin also reproduces the effect of cytokinins on cell enlargement, on the extrusion of hydrogen ions, and on the transmembrane potential in isolated cotyledons (Marre *etal* 1974)

The nitrogenous compounds thiourea and nitrate alleviated germination inhibition completely in lower salinities (25- 50 mM NaCl) and partially in higher salinities (100 mM NaCl). Several studies have reported that the increase in NaCl concentration affected protein synthesis through the inhibition of some enzymes that regulate the process of protein synthesis (Ashraf et al. 1995, 2002; Prado et al. 1995, 2000). However, the prolonged exposure of seeds to high NaCl concentrations has an irrecoverable effect on germination level, germination velocity and RNA synthesis (Siddiqui 2006). Siddiqui (2006) reported that the recovery of seed germination in nitrate and thiourea usually result in restoration of metabolic activity. In addition, thiourea counteracts the effects of increased ABA and reduced the level of cytokinin in plant tissues exposed to drought, which is induced by water stress, salinity, or high temperatures (Kabar and Baltepe 1989). Furthermore, nitrogenous compounds are known to promote germination by acidification

and Softening of cell walls or by activating the pentose phosphate pathway (Esashi et al. 1979).

4.9. Compositional Analysis

The compositional data *of C. conglomeratus* seeds indicate that the seeds are very nutritious and rich in fat. The high values of fat and protein in sample collected from Zakhir area could be due to the availability of treated water from a close sewage treatment plant as the area sometimes gets wet by the over flowing water from the treatment plant.

The mineral composition of seeds collected from different areas. It can be seen from the table that seeds are rich in nutrient minerals especially potassium, magnesium, phosphorous, iron and zinc. There are some differences between different habitats .The high values in the sample collected from Zakhir area could be due to the availability of treated water from a close by sewerage treatment plant as the area sometimes gets wet by the over flowing water from the treatment plant.

The fatty acid profile of the *C. conglomeratus* oil indicates that the oil is very close to sunflower seed oil. Oleic acid is predominant fatty acid which amounts to about 74 % of the total fatty acids. It is a monounsaturated omega-9 fatty acid found in various animal and vegetables. The other highest percentage was for Lenoleic acid about 13.1 %. It is one of the polyunsaturated fatty acid (PUFA), especially omega-6 fatty acid, it also belongs to the group of essential fatty acids (EFAs), which means that it cannot be synthesized by the human body, but must be obtained in the diet.Interest in the PUFA, as health-promoting nutrients has expanded in recent years. Many literatures illustrate the benefits of PUFA in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finley & Shahidi,

2001). Finally the seed contains about 7.5 % Palmitic acid and only traces for other fatty acids.

It is concluded that C. conglomerates seeds are highly nutritious rich in minerals and essential fatty acids. They are good sources of protein, fat and minerals for the grazing animals such as camels, deers and gazelles.

4.9.1 SFE Extraction of C.conglomeratus's oil

Pressure effect:

At all temperatures, the extraction yield increased with pressure. This is attributed to the effect of pressure on CO_2 density; higher pressures result in higher CO_2 densities, leading to higher solvating power of CO_2, which results in higher solubility of oil and thus higher extraction yields. Similar findings were found in a study conducted by Ugur Salgın (2004) on jojoba seed ,which was carried out at pressures of 25, 35 and 45MPa, and two temperatures of 70 °C and 90 °C. An increase in operating pressure resulted in increasing the density of the supercritical fluid and a subsequent increase in its solvating power, which results in higher extraction yield.

Temperature effect:

The effect of temperature on the extraction yield was varried at different pressures. The varying effect of temperature on the extraction yield may be due to two competing factors (density of the supercritical CO_2 and volatility of the solute), which depend on the temperature in opposite ways. Higher temperatures increase the volatility of solutes and improve their solubility and extraction. On the other hand, density of supercritical CO_2 decreases with increasing temperature, reducing the solvating power of CO_2 and thus reducing the solubility and extraction efficiency.

Similar results have been reported by other investigators. Ugur Salgın conducted experiment on sunflower oil, at the pressures of 20, 30, 40, 50 and 60 MPa; the

temperatures of 40, 60 and 80 °C, and showed that the solubility of sunflower oil in supercritical CO_2 increased slightly with temperature at higher pressures (above 30 MPa). However, solubility of sunflower oil in supercritical CO_2 at the pressure of 20MPa decreased significantly with temperature. This is due to the fact that an increase in temperature at constant pressure leads to a drop in CO_2 density as well as an increase in the vapor pressure of solutes. The drop in CO_2 density is substantial at pressures near the critical point, resulting in a drop in solubility. However, at higher pressures the drop in CO_2 density due to the similar temperature increase is small, which is overcome by the vapor pressure increase and the net effect is a solubility increase (Bozan, *etal* 2003).Similar solubility behavior has been reported for other vegetable and seed oils (Ozkal *etal* 2005).

CHAPTER 5

CONCLUSION

5. CONCLUSION

Few species stand the instability of sand dunes and low nutrient content of its soils. *Cyperus conglomeratus* is one of these species and considered to be the most abundant species on the sand dunes in the UAE.

The present study aimed at assessing seed dormancy, germination requirement, salinity and tolerance during the critical germination stage, the best time for seed collection, the effect of storage on germination behavior of *C. Conglomeratus*. The study also aimed at determining the composition of seeds (moisture, ash content and minerals, oil content, protein, carbohydrates and fiber content) as well as fatty acid composition of oil to evaluate its quality for food/feed applications. The yield and quality of oil obtained by supercritical fluid extraction (SFE) and solvent extraction techniques also assessed during the study.

We conclude from this study that none of studied dormancy regulating substances improved seed germination. Also there was significant effects for maternal habitat, time of seed collection, seed storage and both light and temperature of seed incubation and their interaction on both germination percentage and rate. Seeds of *C. conglomeratus* produced on dry sand dunes (Abu Dhabi and Dubai) produced higher germination percentages whereas those produced in Al-Ain showed significantly lower germination. The lowest germination was for Liwa .

In addition Germination in light was significantly greater than it in dark, especially at higher temperature and germination at higher temperatures was significantly greater than that at lower temperatures, which ensures that they will germinate successfully on or near the soil surface when other conditions are suitable for seedling emergence.

For the effect of salinity the germination was greatly reduced in 25 mM NaCl and completely inhibited in 50 mM NaCl. Its tolerance was greatest in darkness at higher

temperatures. For all salinities, no seeds were recovered at the lower temperatures and recovery germination percentage was significantly greater at higher temperatures. Fusicoccin, GA3 and kinetin, completely alleviated salinity induced dormancy in all salinity levels. Nitrate and thiourea completely alleviated the germination inhibition in lower salinities, but partially alleviated it in 100 mM NaCl.

The effect of Polyethylene osmotic pressure on the germination of *C. conglomeratus* seeds was significant, which indicates that the germination inhibition in saline solutions is more likely due to osmotic effect, rather than ion toxicity.

Final germination of fresh seeds of *C. conglomeratus* was significantly greater, compared to it for seeds stored for 15 months. This result indicates that storage either induced a secondary dormancy or resulted in viability loss of the seeds.

Interestingly there was no compositional analysis data available for the seeds of this plant. The present study showed that the seeds of this plant contain moisture 2.51%, ash – 7.74%, fat – 29.5% and protein – 13.5%. Also its rich in oil and the fatty acid profile of the soxhlet extracted fat was determined by capillary gas chromatography and found to be: C10:0 – 0.10, C12:0 – 0.10, C14:0 – 0.16, C16:0 – 7.47, C16:1 – 0.12, C18:0 – 3.52, C18:1 – 73.8, C18:2 – 13.14, C18:3 – 0.56, C20:0 – 0.12, C20:1 – 0.27, C22:0 – 0.39, C22:1 – 0.17. Lipids were also extracted using supercritical fluid extraction (SFE) and SFE conditions of 50 °C and 400 bar gave maximum yield of 28.6%. The fatty acid profile of SFE oil was similar to that of the soxhlet extracted oil. The mineral composition (mg/kg) was found to be as: Na: 297, K: 3029, Ca: 896, Mg: 2303, Fe: 191, P: 3491, Cu: 9.57 and Zn: 53.3. Heavy metal contaminants (mg/kg) – Pb: 0.23, Cd: 0.14 were found to be within acceptable limits.

The study concludes that C. *conglomeratus* seeds are highly nutritious, rich in fat, protein and essential minerals such as potassium, phosphorous and iron. The seed oil is also rich in unsaturated fatty acids. The seeds could serve as good animal feed. The presence of quality fat in higher quantities make the seeds also suitable for poultry feed as high amounts of fat and protein are required for the quick growth of broilers.

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كان إنبات البذور بشكل عام في الضوء أكثر منه في الظلام مما يدل على قدرة هذه النبتة على النمو إما على سطح التربة أو قريبا جدا من السطح.

أظهرت النتائج قلة إنبات هذه البذور في المحلول الملحي 25مل مول من كلوريد الصوديوم و انعدامها تماما في المحلول 50 مل مول .بينما أظهرت البذور غير المعالجة بمحاليل الأملاح نسبة عالية من الإنبات في درجات الحرارة المختلفة تحت ظروف الظلام والإضاءة. إلا أنها استعادت قدرتها على الإنبات بعد نقلها إلى ماء مقطر. أظهرت البذور قدرة مثلى لاستعادة قدرتها على الإنبات في درجات الحرارة العالية مقارنة بدرجات الحرارة المعتدلة والمنخفضة. وهذا يدل أن قدرة استعادة الإنبات للبذور الخاضعة لتراكيز ملحية مختلفة تحت درجات الحرارة العالية.

بذور الثندة التي انخفضت نسبة إنباتها بسبب المحلول الملحي استعادت قدرتها على الإنبات تماما باستخدام حمض الجيريليك، و النترات والكاينتين والثيوبوريا، و لم تتأثر الفيوسيكوسين.

كما أظهرت النتائج انه نسبة الإنبات للبذور الحديثة أكبر منها للبذور المخزنة لمدة 15 شهرا وقد يكون ذلك بسبب فقدانها للقدرة على الإنبات نتيجة ظروف التخزين.

لم تتوفر اي معلومات عن التركيب الكيميائي لهذه البذور وقد أظهرت نتائج الدراسة الحالية أنها تحتوي على نسبة رطوبة منخفضة بنسبة 2.5 و نسبة كبيرة من الزيت تصل 29.5% و هي نسبة نادرة بالنسبة لنبتة صحراوية ونسبة 13.5 من البروتين. كما أنها تحتوي على نسبة عالية من المعادن المتنوعة مثل الصوديوم و البوتاسيوم والحديد و الماغنيسيوم و الكالسيوم. بالإضافة إلى نسب قليلة جدا من المعادن الثقيلة .

أظهرت هذه الدراسة انه نبتة الثندة لها قيمة غذائية عالية جدا و تحتوي على نسبة جيدة من الأحماض الدهنية الغير مشبعة , مما يساعد هذه النبتة بأن تكون مصدر غذائي قيم للحيوانات و الدواجن.

الملخص باللغة العربية

يعتبر نبات الثندة (*Cyperus conglomeratus*) من النباتات القليلة التي تتحمل تفكك و حركة الكثبان الرملية وكذلك تمكنها من الاستقرار في مثل هذه البيئة. كما انه تم تصنيفها ضمن النباتات الرعوية المقاومة للجفاف خلال فترة النمو الخضري ومرحلة التكاثر، كما دلت بعض الملاحظات أن بذورها قد تحتوي على كمية جيدة من الزيوت.

بالرغم من الأهمية البيئية لنبات الثندة إلا انه يفتقر إلى الكثير من الدراسات حول ظاهرة الكمون وإنبات البذور والعوامل البيئة التي تؤثر على عملية الإنبات وكذلك حول نوعية و كمية الزيوت التي تحتويها البذور.

تم التركيز في هذا البحث على دراسة الكمون الطبيعي في البذور بالإضافة إلى متطلبات الإنبات من الحرارة والإضاءة ومقاومة للجفاف والملوحة وكذلك تم تحديد تأثير منظمات النمو على إنبات البذور ، بالإضافة إلى دراسة تأثير الخزن وبيئة النبات الأم(البيئة التي تم فيها نضج البذور قيد التجربة) على فترة الكمون ومتطلبات الإنبات و دراسة التركيب الكيميائي للبذور وللزيت الذي تحتويه.

خضعت البذور الحديثة النضج والمخزونة لفترات متفاوتة للإنبات تحت ظروف مختلفة. أيضا أخضعت البذور لتراكيز مختلفة من الضغط الأسموزي باستخدام تراكيز مختلفة من الملح (كلوريد الصوديوم) وايثيلين الجليكوحل المتعدد 6000 (PEG 6000). تمت معظم تجارب الإنبات تحت درجات حرارة وإضاءة مختلفة.

أظهرت بذور نبات الثندة كمون طبيعي قليل حيث انبتت حوالي 84 % من البذور حديثة النضج. كما إن غالبية المواد الكيميائية المنظمة للكمون لم يكن لها تأثير ايجابي على زيادة نسبة الإنبات.

ولقد أظهرت النتائج أيضا أن بيئة الأم و تخزين البذور لها أثر ايجابي على نسبة و سرعة الإنبات. البذور التي جمعت من مناطق الختم و النهضة و دبي أعطت إنبات أكثر و أسرع مقارنة بالبذور التي - ، من منطقتي المناصير و المنطقة الصناعية بالعين .و كانت البذور التي جمعت من منطقة ليوا أقل البذور إنباتا.