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Production of nursery-reared seedlings of the gray mangrove *Avicennia marina* under laboratory conditions

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ABSTRACT
Avicennia marina (Forssk.) Vierh. is the exclusive mangrove plant species that is naturally distributed along the Egyptian coast of Gulf of Aqaba, Red Sea. It has the broadest distribution, both latitudinally and longitudinally compared to any other mangrove species. In spite of the fact that mangrove forests usually grow in the intertidal zone, very few species such as *Avicennia marina* can grow in a truly saline environment. The aim of this study is to investigate germination of *A. marina* seeds and subsequent growth parameters of their seedlings in response to three different salinity levels composed of tap water, 50 % and 100 % seawater (0, 21, 42 ‰ salinity). Experimental results indicate that 21 ‰ salinity (50% seawater) has the highest germination percentage (%), leaf size (cm^2), internode length (cm) and plant height (cm). Moderate germination percentage, leaf morphology and stem growth was recorded for the tap-water treatment. The high salinity treatment of 42 ‰ (100 % seawater) inhibited seed germination. These results could be used in mass production of seedlings and saplings during the process of restoration of this threatened species.

KEYWORDS: germination; leaf morphology; water salinity; Gulf of Aqaba; Red Sea, Sinai.

INTRODUCTION

Mangroves are usually found in tropical and sub-tropical marine inter-tidal zones (Tomlinson 1994) and are usually dominated by halophytic woody species (Suarez *et al*. 1998). Mangrove forests act as the borderline between land and sea. Their habitats are ecologically vital. They function as natural nutrient filters and recyclers, aid in floodwater improvement and protect coastlines from erosion, storm damage and wave action by acting as buffers trapping alluvial materials and thus stabilizing land elevation by sediment accretion that balances sediment loss (Balakrishna 1995). The ability to use seawater is one striking attribute of mangrove species. However, mangrove forests are threatened ecosystems (Duke 1990). Reclamation of mangrove land for agriculture, aquaculture, horticulture, human settlement, industries and salt production are some of the reasons for the present situation of reduction in mangrove forests all over the world. Coastal pollution with heavy metals and petroleum are also a threat to mangrove plants and their associated flora and fauna (Kairo *et al*. 2001), and they have also declined due to natural disasters (Jimenez 1985). Natural regeneration rates may continuously restore some of the destroyed mangrove areas, but it is rare for new mangrove patches to arise (Saenger & Siddique 1993).

Avicennia marina (Forssk.) Vierh. (Avicenniaceae) is one of the most widespread mangroves in tropical and subtropical regions (Tomlinson 1994). It usually persists in a wide range of salinity levels and survives well in high salinity habitats, showing a distinctive shrubby form. A steady infusion of fresh water is essential to lessen the level of salinity in soil and water. Any reduction in the supply of fresh water results in an increase in salinity, which restricts mangrove growth (Jeminez 1990).

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Although seedling establishment is critical in the life cycle of all seed plants, it is rendered difficult for mangroves because of the unstable and variable substrates and the tidal influence within mangals (Tomlinson 1994). The hydrological regime is particularly important in controlling the survival and subsequent growth of the mangrove seedlings (Murray *et al*. 2003).

The present study aims to develop a simple and inexpensive laboratory method for the production of seedlings intended for restoration of *A. marina*. It examines the influence of three different salinity levels 0, 21 & 42 ‰ on germination and growth in order to determine the optimum salinity level.

MATERIALS AND METHODS

Seeds of the gray mangrove *Avicennia marina* var. *marina* were collected twice in April and November 2002 from naturally growing trees distributed along the Egyptian coast (Red Sea at El-Nabq, Gulf of Aqaba): voucher specimens from the mature plant were deposited in the Botany Department Herbarium, Suez Canal University, Ismailia, Egypt. Mature seeds were separated easily from the parent trees with a slight hand twist without the calyx (Kairo 1995). After field collection, seeds were kept in moist plastic bags and protected from direct sunlight until transported to the lab at Ismailia.

A preliminary germination experiment was conducted on seeds collected in April 2002. The importance of pre-treatment conditioning such as sowing in tap water, 50 and 100 % seawater was attempted first in order to inform the design of the full experiment. Seeds of *A. marina* were planted in pots with a clay:sand mixture (1:1) in greenhouse conditions (light radiation 100-150 μmol $m^{-2}s^{-1}$, temperature 30 ± 5°C during the day and 20 ± 5°C at night). About fourteen seeds per treatment were grown at three salinities (0, 21, 42 ‰) maintained for 6 months and watered every other day; the germination percentage was determined. Continuous morphometric measurements were performed for seedlings grown in various seawater concentrations over a period of twelve months. About 24 replicates of plant parts were measured for various growth parameter from each treatment. Some seeds were planted in clear glass jars on cotton and watered with 0, 21, or 42 ‰ salinities in order to investigate root growth and to derive the root-shoot ratio.

Leaf morphology and stem growth were used as characteristic distinctions between *A. marina* plants grown at different salinity treatments. Growth parameters including plant height (cm), stem diameter (cm), length of leaf blade (cm), average blade width (cm), leaf size (cm^2), number of nodes, length of internodes (cm) and number of surviving leaves on shoots were continuously measured over a period of 6 months (according to Duke 1991). From these data, relative growth rates (RGR) for both plant height (RGR_H), and leaf area (RGR_A), node and leaf production per shoot per year, as well as the root/shoot ratio were determined. The mean relative growth rate was calculated according to the equation $RGR = (H_2 - H_1)/ (t_2 - t_1)$, where H_2 & H_1 are plant height at the beginning and end of the experimental period, and $t_2 - t_1$ is the time gap (Hwang & Chen 2001).

Differences among the three salinity treatments were analyzed using the one-way ANOVA test. Statistically significant differences between treatments were assessed using Tukey's HSD significant difference (Lentner & Bishop 1986; Zar 1984). Mean values are given ± standard errors.

RESULTS

The preliminary experiment showed that sowing *A. marina* seeds for 48 h is essential in promoting maximum germination rates. Sowing helps to remove the seed coat and aids in the emergence of the radicle before planting seeds above the surface of the soil. Generally the environmental conditions were satisfactory. Large *A. marina* seeds were more likely to survive, and therefore they were used in the main experiment. Changes in germination rates and plant morphology were noticed as the main reaction to the salinity level (Figure 1 A & B).

Seeds of *A. marina* are viviparous, since the embryo usually germinates rapidly on release. Their germination is usually epigeal with an extending hypocotyl, and the radicle contributes also to its extension. Comparison of the germination percentage (%) of seeds grown at different salinity levels (Figure 2) shows a significant (p = 0.000) inhibitory effect of high salinity (42 ‰) on the germination process. Less than 20 % germination was achieved under high salinity, much lower than for fresh water or 21 ‰ salinity: 21 ‰ salinity produces the highest germination rate. Seedling emergence also is significantly affected by high salinity (Figure 3). Splitting of the pericarp and separation of the cotyledons were earlier in both fresh water and 21 ‰ salinity treatments. Prominent delays in the separation of cotyledons as well as shoot emergence were obvious for seedlings watered by 42 ‰ salinity (Figure 3).

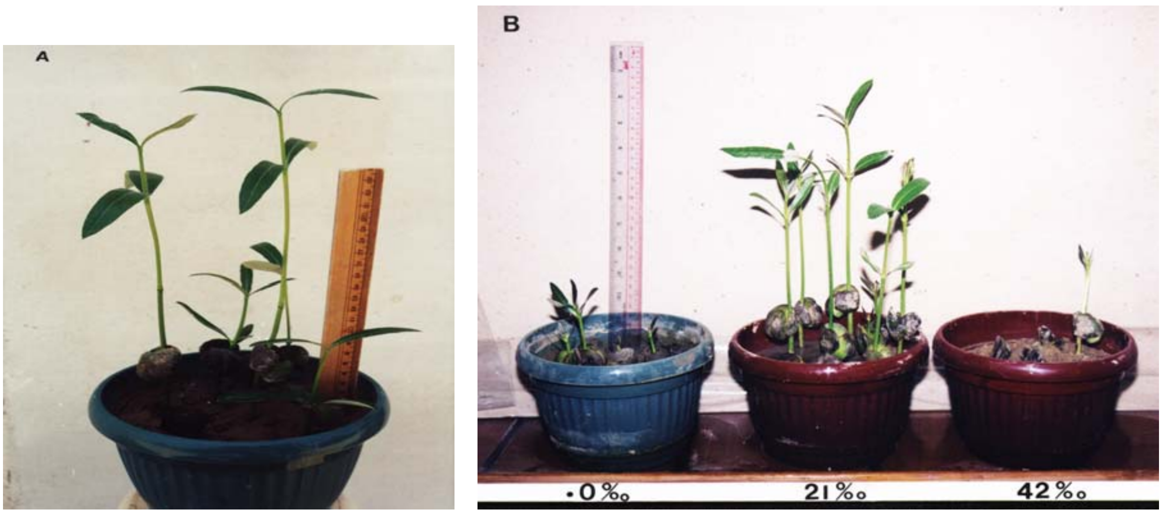

Figure 1: (A) Indoor growth of *Avicennia marina*, (B) *A. marina* plants growing at 0, 21, 42 ‰ salinities.

The shape of the leaf blade was constant among the three salinity treatments: all leaves had narrowly oblong blades, with parallel and entire margins. The length to breadth ratio was 4:1in both saline treatments, but only 3.5:1 for the tap-water treatment. Leaves are generally green on the upper surface and gray beneath with an obtuse base and short petioles (about 1 cm). Two leaves are usually produced at each node. Leaves are decussately arranged along the stem. Leaf morphology was investigated through determination of four parameters including length and width of blade, length of petiole and leaf size (Table 1). The highest mean values for many of these variables was recorded for 21 ‰ salinity treatment, and these differences were usually statistically significant.

The number of leaves surviving on stems at high salinity after 6 months is less than the other treatments: leaves fall down as a consequence of high salt accumulation in leaf structures.

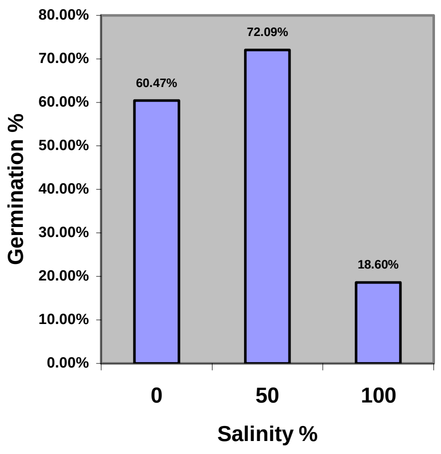

Figure 2: Germination percentage % of *Avicennia marina* seeds germinated at different salinity levels.

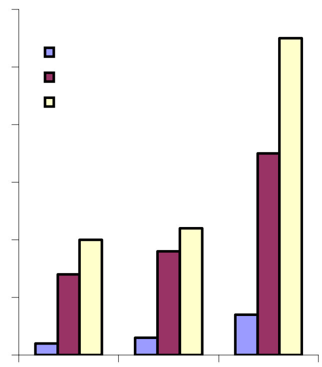

Figure 3: Growth of early juvenile stages of *Avicennia marina* seedlings grown at different salinities.

Table 1: Mean values of growth parameters recorded for *Avicennia marina* plants grown for 6 months in different seawater dilutions. Mean values followed by different letters are significantly different at the $P < 0.05$ level, based on Tukey's HSD. ** = significantly different at the 0.01 level, *** = significantly different at the 0.001 level, NS = not significant.

	Seawater %			
	0	**50**	**100**	**F-ratio**
Growth parameters	**Mean**	**Mean**	**Mean**	
Length of blade (cm)	6.16 ± 0.25a	7.51 ± 0.21b	6.63 ± 0.27a	12.71***
Width of blade (cm)	1.75 ± 0.04	1.85 ± 0.06	1.70 ± 0.14	1.18 NS
Leaf petiole length (cm)	0.90 ± 0.03	0.53 ± 0.03	0.90 ± 0.04	1.21 NS
Leaf size (cm^2)	10.94 ± 0.34a	14.58 ± 0.41b	11.59 ± 0.34a	12.79***
Plant height (cm)	26.29 ± 3.04a	37.17 ± 1.21c	30.5 ± 2.25b	10.07***
Number of nodes	3.06 ± 0.21a	3.42 ± 0.15a	2.63 ± 0.21b	3.57**
Internodal extension (cm)	9.04 ± 0.38a	10.56 ± 0.33b	12.16 ± 0.38c	10.37***
Stem diameter (cm)	0.27 ± 0.02a	0.39 ± 0.01b	0.26 ± 0.01a	12.20**
Surviving leaves on shoots	5.2 ± 0.35a	4.4 ± 0.21b	4.17 ± 0.19b	7.12***

Stem growth of mangrove is usually described in terms of two distinct parameters, node production and internodal extension (Figure 1 A). The stem is glabrous, with a circular cross-section. Node production along the stem per shoot per year (Table 2) was about 6 for both fresh water and 21 ‰ salinity treatments, while only about 5 for the 42 ‰ salinity treatment. Internodal extension shows significant differences among treatments (Table 1), and is responsible for plant height. Both high and medium salinities produced long internodes. Usually internode length is variable along *A. marina* stem. The hypocotyle node develops the tallest internode (11.6 – 16.5 cm), and then internode length decreases up the stem (Figure 1 A). Branching of the stem usually occurs after the production of four nodes. One or two branches were produced at every node. The number of nodes and leaves produced per shoot per year as well as number of surviving leaves on shoots are also morphological characters affected by high salinity (Tables 1-2), but the root/shoot ratio did not differ among treatments.

The height of *A. marina* seedlings was significantly affected by the salinity level (Table 1). Medium salinity produced the tallest plants (30 - 55.5 cm) followed by those grown at high salinity (17 – 40 cm).

Table 2: Mean values of relative growth parameters of *Avicennia marina* plants grown at different salinities.

Growth parameters	Seawater % 0	50	100
Relative growth rate (height) mm/ mm/ day	2.83×10^{-3}	3.67×10^{-3}	3.33×10^{-3}
Relative growth rate (leaf size) cm^2 /cm^2/ day	1.51×10^{-3}	2.1×10^{-3}	1.7×10^{-3}
Root / shoot ratio	0.28	0.36	0.25
Node production (nodes/ shoot/ year)	6	6	5
Leaves production (leaves/ shoot/ year)	12	12	10
Range of survival leaves/ shoot/ year	10 -12	8 -10	4 -6

DISCUSSION

Mangrove forests act as the interface between land and sea. They help to protect and stabilize coastlines from erosion, storm damage and wave action by acting as buffers, trapping alluvial materials that form together with the fallen leaves the base of detritus food chains (GEF 1998 a & b). The richness of mangal-associated flora and fauna of the Red Sea coasts (Gab-Alla 2000) signifies the substantial diversity of these unique mangrove forests that represent the northernmost limit for mangroves (GEF 1998 a & b). In addition, conservation of mangrove ecosystems may contribute directly to securing seafood resources in Egypt (Kairo *et al*. 2001). The relationship between mangrove and fishery production has been documented for many areas all over the world (Kairo *et al*. 2001). Production rates of both fish and shrimp decline where mangroves have been removed (Wackemagel & Rees 1996). Mangroves of the Egyptian coasts of Gulf of Aqaba and the Red Sea are suffering from both ecological and environmental stress (GEF 1998 a & b). They gradually being degraded and destroyed due to their exploitation through wood cutting and animal grazing, exposure to phyto-pathological and physiological diseases and pollution (Galdstone *et al*. 1999). Additionally, increasing coastal population and associated development as well as the poor coastal-zone planning and limited technical expertise may contribute to declining mangrove areas of the Egyptian coastal sea shores of the Red Sea (Galdstone *et al*. 1999).

Recently, there is a growing interest for the conservation and restoration of the damaged areas of mangrove forests in the Egyptian coasts by the Egyptian Environmental Affairs Agency in combination with the Food and Agriculture Organization of the United Nations. This has encouraged biologists to study the feasibility of restoring the gray mangrove under local environmental conditions.

The main driving force of this study is the knowledge that mortality of most mangrove species is especially high during the establishment phase, while root systems are still insufficiently developed. Consequently, seedlings should be raised as long as possible in a nursery before transplanting to the field. The current study shows that intermediate salinities are best for *A. marina* and growth is inhibited at full-strength seawater. The suitability of a 50%-seawater treatment as the best watering system for experimental conditions agrees with other results for *A. marina* (Ziche *et al*. 2000), *Rhizophora mucronata* (Vistro & de Soyza 2001) and *Ceriops tagal* (Aziz & Khan 2001). Half-strength seawater produced the highest germination rate, the most efficient seedling development and the best subsequent plant growth, compared to both fresh water and saline treatments.

A. marina is a salt-tolerant plant species (Youssef & Ghanem 2002) and seeds used for the experiment are developed within the fruits of the mother tree, which may contribute to their adaptation to saline environments (Duke *et al.* 1998). Despite those facts, high salinity levels normal for natural environments of the Red Sea was not optimum either for seed germination or for raising seedlings under experimental conditions. We could conclude from this study and some other studies reported in the literature, that experimental pots filled with soil may hold salts more than continuously running seawater in natural environment (Hwang & Chen 2001).

Development of the hypocotyl involves salt loss, and hence does not contribute to the accumulation of salts in various plant organs (Zheng *et al*. 1999). Generally, salts accumulate in plant leaves during the process of leaf production. Older leaves that accumulate extra salts in their structures are prone to drying and abscising. It was noticed from this study that six-month-old *Avicennia* plants watered with 42 ‰ and 21 ‰ salinity regularly abscise some of their old leaves. These observations account for the present figures for the number of surviving leaves on stems, which is less than the leaf production rate. Starting from six-months old, plants subjected to the freshwater treatment produced leaves with dark and dry tips, but these leaves do not detach from their stems afterwards. More than 80 % of seeds watered with high saline water were completely inhibited and failed to germinate, representing a great loss of seeds. It is important to note that plants watered with fresh water under experimental conditions may not survive in a natural saline environment (Vistro & de Soyza 2001).

In conclusion, the growth of *Avicennia marina* responds to salinity by changes in growth rates. Both high and medium salinity levels at 42 ‰ and 21 ‰ produced tall plants with large leaf sizes that are important for general plant development. Comparing germination percentage and seedling emergence of *A. marina* seeds grown at different salinity levels show significant inhibitory effect of high salinity, and that medium salinity levels were best. In general, the study shows that greenhouse production of *A. marina* plants is simple and successful, which is essential for *in situ* restoration programs. Enormous efforts should be devoted to mangrove restoration to conserve such valuable genetic resources (Melville & Burchett 2002) and associated mangal biodiversity, provide employment to local population, guard fragile tropical coastlines and secure seafood resources in Egypt.

REFERENCES

Aziz I & Khan MA (2001). Experimental assessment of salinity tolerance of *Ceriops tagal* seedlings and saplings from the Indus delta, Pakistan. *Aquatic Botany* 70: 259-268.

Balakrishna P (1995) Evaluation of intra-specific variability in *Avicennia marina*, using RAPD markers. *Current Science* 69 (11): 926-929.

Bousquest-Mélou A & Thrérése Fauvel M (1998). Inter-specific variation in the concentration of two iridoid glucosides in *Avicennia* L. (Avicenniaceae Endl.). *Biochemical Systematics & Ecology* 26: 935-940.

Duke N (1990) Phenological trends with latitude in the mangrove tree *Avicennia marina. Journal of Ecology* 78: 113-133.

Duke N (1991) A systematic revision of the mangrove genus *Avicennia* (Avicenniaceae) in Australasian. *Australian Systematic Botany* 4: 299-324.

Duke NC, Ball MC & Ellison JC (1998) Factors influencing biodiversity and distributional gradients in mangroves. *Global Ecology & Biogeography Letters* 7: 27-47.

Gab-Alla AA-FA (2000) Biodiversity and distribution of epiphytes community associated with pneumatophores of the mangal *Avicennia marina* (Forssk.) Vierh., along Egyptian Red Sea Coast. *Egyptian Journal of Aquatic Biology & Fisheries*. 4 (1): 179-196.

GEF (1998a) Egyptian Red Sea Coastal and Marine Resource Management Project. Report 5: Red Sea Coastal and Marine Protected Area Strategy. Part 1: Strategy Development. Pp. 50-51.

GEF (1998b) Egyptian Red Sea Coastal and Marine Resource Management Project. Report 5: Red Sea Coastal and Marine Protected Area Strategy. Part 2: Ecosystem Guidance & CMPA Action Plans. 104-111.

Gladstone W, Tawfiq N, Nasr D, Andersen I, Cheung C, Drammeh H, Krupp F & Lintner S (1999) Sustainable use of renewable resources and conservation in the Red Sea and Gulf of Aden: issues, needs and strategic actions. *Ocean & Coastal Management* 42: 671-697.

Hwang Y-H & Chen S-C (1995) Anatomical responses in *Kandelia candel* (L.) Druce seedlings growing in the presence of different concentrations of NaCL. Bot. Bull. Acad. Sin. 36: 181-188.

Jimenez JA (1990) The structure and function of dry weather mangroves on the Pacific coast of Central America, with emphasis on *Avicennia bicolor* forests. *Estuaries* 13: 182-192.

Jimenez JA, Martine R & Encamocion L (1985) Massive tree mortality in Puerto Rican mangroves forests. *Carribean Journal of Science* 21: 75-78.

Kairo JG (1995) Artificial regeneration and sustainable yield management of mangrove forests in Gazi Bay, Kenya. M.Sc. Thesis, University of Nairobi, Nairobi, Kenya, pp.116.

Kairo JG, Dahdouh-Guebas F, Bosire J & Koedam N (2001) Restoration and management of mangrove systems - a lesson for and from the East African region. *South African Journal of Botany* 67: 383-389.

Lentner M & Bishop T (1986) Experimental Design and Analysis. Valley Book Company, Blacksburg, USA.

Melville F & Burchett M (2002) Genetic variation in *Avicennia marina* in three estuaries of Sydney (Australia) and implications for rehabilitation and management. *Marine Pollution Bulletin* 44: 469-479.

Murray MR, Zisman SA, Furley PA, Munro DM, Gibson J, Ratter J, Bridgewater S, Minty CD & Place CJ (2003) The mangroves of Belize Part 1. Distribution, composition and classification. *Forest Ecology & Management* 174: 265-279.

Saenger P & Siddique NA (1993) Land from the sea: the afforestation program of Bangladesh. *Ocean & Coastal Management* 20: 23-39.

Suaréz N , Sobrado MA & Medina E (1998) Salinity effects on the leaf water relations components and ion accumulation patterns in *Avicennia germinans* (L.) seedlings. *Oecologia* 114: 299-304.

Tomlinson PB (1994) The Botany of Mangroves. Cambridge University Press. New York, USA.

Vistro NR & de Soyza AG (2001) Effects of water salinity on seedling emergence, growth and physiology of *Rhizophora mucronata* in the UAE. Proceedings of the International Symposium on Mangrove & Saltmarsh Ecosystems, pp. 125-130.

Wackemagel M & Rees WE (1996) Our Ecological Foot-Print: Reducing Human Impact on Earth. New Society Publishers, Gabriola Island, BC: Stony Creek, C.T., USA.

Youssef T & Ghanem A (2002) Salt secretion and stomatal behavior in *Avicennia marina* seedlings fumigated with the volatile fraction of light Arabian crude oil. *Environmental Pollution* 116: 215-223.

Zar JH (1984) Biostatistical Analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J., USA.

Zheng WJ, Wang W-Q & Lin P (1999) Dynamics of element contents during the development of hypocotyls and leaves of certain mangrove species. *Journal of experimental Marine Biology & Ecology* 233: 247-257.

Ziche D, Kaser B & Forsteuter M (2000) Growth and photosynthetic parameters of the mangrove *Avicennia marina* under different salinity. Symposium of Cash Crop Halophytes: Potentials, Pilot Projects, Basic and Applied Research on Halophytes and Saline Irrigation 10 years after the ALAIN Meeting under the Patronage of H. H. Sheikh Sultan Bin Zayed Al-Nahayan, May 29 to June 2, Osnabrueck, Germany.

الملخص العربى

إنتاج شتلات من نبات الشورى (المانجروف الرمادي) على نطاق معملي

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يعتبر مجتمع نباتات المانجروف من أثرى النظم البيئية عالميا من حيث الإنتاجية والتنوع البيولوجي. توفر هذه المنظومة البيئية الفريدة أماكن آمنة لتكاثر صغار الكائنات البحرية ومرعى صيد لها. كما توفر بعض المنتجات النباتية مثل الأخشاب، الفحم الحجري، أخشاب التدفئة وكذلك تمد قطاع الصناعة بالمواد الخام الصالحة للصناعات القائمة على السليلوز. ولكن من المؤسف أن غابات نباتات الشورى من أكثر البيئات الطبيعية تعرضا لمخاطر تؤدى إلى النقص في مساحتها وإنتاجيتها.

يهدف هذا البحث إلى دراسة نسبة إنبات بذور نبات الشورى وكذلك نمو البادرات وأطوال النباتات وحجم الأوراق عند إنباتها بالمعمل وريها بثلاث نظم ري تتكون من ماء الصنبور, ماء ذو ملوحة 21 ‰، وآخر ذو ملوحة 42 ‰ . وتؤكد القياسات المختلفة على البادرات والنباتات النامية، لمدة ستة أشهر على تربة تتكون من 1:1 طين: رمل، التأثير السلبي للملوحة العالية على نسبة الإنبات وعلى نمو البادرات والتأثير المثالي للمياه ذو الملوحة المتوسطة 21 ‰ والتي تتفوق أيضا على استخدام ماء الصنبور العذب. إن ري بذور نبات الشورى بمياه نسبة ملوحتها 21 ‰ يساهم في استنبات حوالي 80 % من البذور المستخدمة، كما ينتج نباتات طويلة ذات أوراق أكبر حجما من حيث طول النصل وسيقان ذات عدد عقد أكثر وسلاميات أطول من المعاملات الأخرى. حيث أن الري المعملي للبادرات بماء الصنبور يعطى نسبة إنبات للبذور ونمو للبادرات أقل من المعاملة بنسبة ملوحة متوسطة. أما الري بالماء ذو نسبة ملوحة 42 ‰ يثبط إنبات البذور مما لا يعطى الفرصة للبذور السليمة ذات الحيوية الطبيعية أن تنتج بادرات في أغلب الأحوال. وتساهم هذه الدراسة في إنتاج شتلات نبات الشورى تحت ظروف معملية على نطاق واسع للاستزراع في أماكنها الطبيعية.