



UNIVERSITI PUTRA MALAYSIA

***IMPACT OF SEA WATER, DEAD SEA WATER AND NaCl ON
GERMINATION, ANATOMICAL AND ANTIOXIDANT PROFILE OF
MOLOKHIA (Corchorus olitorius L. Malvaceae)***

AYAT MOHAMMAD MUTLEQ TANEENAH

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By

AYAT MOHAMMAD MUTLEQ TANEENAH

**Thesis Submitted to the School of Graduate Studies, Universiti
Putra Malaysia, in Fulfilment of the Requirements for the Degree of
Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
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High salinity is the most severe environmental stress that effects on seed germination, seedlings, and growth in the life cycle of the plants.–Salinity gave negative effect on plant development performance from germination to reproduction such as a reduction in elongation growth, reduction of leaf expansion and reduced stomatal pores and density. Few studies had been done to determine the effect of Sea water and NaCl on the germination, morphology, physiology, and antioxidant activity on Molokhia, and moreover, no study had been on the effect of Dead Sea water on Molokhia plant. This study aimed to understand the response of Molokhia (*Corchorus olitorius* L.) in different concentration of Dead Sea water, sea water, and NaCl at germination stage., anatomical features and antioxidant activity. Twenty-five sterilized Molokhia seeds were germinated in sterilized Petri dishes and treated with 5 ml deionized water (control) or with Dead Sea water, sea water, and NaCl (3‰, 6‰, 9‰, 12‰) (part per thousand), with four replicates for each treatment and were arranged randomly. Germination percentage, germination index, germination rate, salt tolerance and seed vigor were calculated. This study found the germination percentage was highest in control, 3 ‰ of Sea water and 3 ‰ of Dead Sea water (98.4 %, 93.9 %, 93.2%) respectively, however, by increasing salinity concentration, seed germination delayed and decreased. Whereas, salt tolerance and seed vigor declined with increasing salinity concentration except at 3 ‰ of Dead Sea water. Biomass, stomatal conductance, and anatomical leaves of Molokhia seedling as germinated in three different types of salt were studied. Twenty seedlings were selected randomly. The results showed that seedling growth characteristics and stomatal conductance significantly reduced (ANOVA, $P < 0.05$) except at 3‰ of Dead Sea water. The highest leaf area was achieved with 3‰ of Dead Sea water (7.60 mm²). This study found that

the anatomy of seedlings leaves affected by high concentration of NaCl, sea water, and Dead Sea water (9 to 12) ‰ as salinity concentrations increased, the changes of anatomy and arrangement of cells also increased. Moreover, the arrangement of mesophylls tissues was found in disorder. The amount of chlorophyll *a*, *b*, carotenoid, total phenolic content and reduced glutathione were measured spectrophotometrically of *Molokhia* leaves as responses to Dead Sea water, sea water and NaCl. Around 5g of fully expanded leaves were harvested randomly. The concentration of total phenolic compound and carotenoids increased when salinity increased in all treatments which 3‰ of Dead Sea water showed the highest carotenoids (1.10 mg/ml). However, total chlorophyll, chl *a* and chl *b* decreased whilst salinity concentration increased except in 3‰ (4.70 mg/ml) and 6 ‰ (4.20 mg/ml) of Dead Sea. Reduced glutathione (GSH) is most important molecules in plant as stress marker, 3‰ and 6‰ of Dead Sea water shows the highest GSH 20 µmol/g.FW and 15 µmol/g.FW respectively. The findings of this study revealed that the salt tolerance during germination stage expedient to be used 3‰ of Dead Sea water for germination *C.olitorius* seed due to increase the biomass of seedling and leaf area exists. Therefore, more study should be conducted on *Molokhia* plant to understand the ecophysiological strategies for the survival under salinity environment conditions especially under 3‰ of Dead Sea water.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN AIR LAUT, AIR LAUT MATI DAN NaCl KE ATAS
PERCAMBAHAN, ANATOMI DAN PROFIL ANTIOKSIDAN MOLOKHIA
(*Corchorus olitorius* L, Malvaceae)**

Oleh

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Kemasinan memberi kesan negatif dari peringkat percambahan hingga ke peringkat pembiakan pokok. Kajian ini dilakukan untuk mengkaji kesan air laut mati, air laut (sumber: Port Dickson) dan NaCl pada kepekatan yang berbeza terhadap percambahan biji benih, morfologi, fisiologi, dan aktiviti antioksidan Molokhia. Sebanyak 25 biji benih Molokhia yang telah disterilkan disemai ke dalam piring petri dan dirawat dengan 5 ml air ternyahion (sebagai kawalan) atau dengan air laut mati, air laut, dan NaCl (3 ‰, 6 ‰, 9 ‰, 12 ‰). Setiap rawatan mengandungi 4 replikasi dan disusun secara rawak. Peratusan percambahan, indeks percambahan, kadar percambahan, kadar toleransi terhadap garam dan vigor benih telah dikira. Kajian ini mendapati peratus percambahan adalah paling tinggi pada kawalan, diikuti dengan 3 ‰ air laut dan 3 ‰ air Laut Mati (98.4%, 93.9%, 93.2%). Walaubagaimanapun, peningkatan kemasinan menyebabkan percambahan benih semakin lambat dan berkurangan. Kadar toleransi garam dan vigor benih juga menurun dengan peningkatan kepekatan kemasinan kecuali pada 3 ‰ air Laut Mati. Biomas, aliran stomata, dan perubahan anatomi anak benih Molokhia sebagai gerakbals terhadap kemasinan juga dilakukan. Sebanyak 20 anak benih dipilih secara rawak. Hasil kajian mendapati bahawa pertumbuhan dan aliran stomata menurun (ANOVA, $P < 0.05$) kecuali pada 3 ‰ air Laut Mati. Keluasan daun paling tinggi didapati pada rawatan 3 ‰ air laut mati (7.60 mm²). Kajian menunjukkan perubahan anatomi dan susunan sel-sel daun sangat ketara pada kepekatan NaCl, air laut, dan air Laut Mati yang tinggi (9 - 12‰). Selain itu, susunan sel-sel dan tisu daun mesofil adalah dalam keadaan tidak teratur. Kandungan klorofil, kandungan sebatian fenolik dan kandungan antioksidan pada Molokhia yang dirawat dengan air laut mati, air laut dan NaCl juga dilakukan. Jumlah klorofil a, b, karotenoid, kandungan fenolik dan glutathion diukur menggunakan

spektrofotometrik. Kandungan sebatian fenolik dan karotenoid meningkat apabila kepekatan kemasinan tinggi dimana 3 ‰ air laut mati menunjukkan karotenoid tertinggi (1.10 mg/ml). Walaubagaimanapun, jumlah klorofil *a* dan *b* menurun dalam kepekatan yang tinggi kecuali 3 ‰ (4.70 mg/ml) dan 6 ‰ (4.20 mg/ml) air Laut Mati. Penurunan kandungan Glutathion (GSH) digunakan sebagai penanda terhadap sesuatu tegasan. Hasil kajian ini mendapati 3 ‰ dan 6 ‰ air laut mati menunjukkan kandungan GSH yang tertinggi (20 µmol/g.FW dan 15 µmol/g.FW). Sebagai kesimpulan, kajian mendalam perlu dijalankan ke atas *Molokhia* untuk memahami strategi ekofisiologi untuk hidup di dalam persekitaran kemasinan yang tinggi terutamanya di bawah 3 ‰ air Laut Mati. Di samping itu, pada 3 ‰ air laut mati sesuai untuk dimanfaatkan bagi percambahan bijibenih *Corchorus olitorius* L. disebabkan oleh peningkatan dalam biojisim anak benih dan keluasan daun.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
‰	Salinity symbol
° C	Degree celsius
DS	Dead sea water
S	Sea water
NaCl	Sodium chloride
FW	Fresh weight
DW	Dry weight
LA	Leaf area
GP	Germination percentage
GI	Germination index
GT	Germination rate
SV	Seed vigor
ST	Seed tolerance
μmol	Micromole
seeds day ⁻¹	Seeds per day
G	Gram
mg	Milligram
cm	Centimeter
g/area	Gram per area
mm	Millimeter
mm ²	Millimeter square
μm	Micrometer
ml	Milliliter
w/v	Weight per volume
ppt	Part per thousand
CRD	Complete random design

$\mu\text{mol m}^{-2} \text{s}^{-1}$	Light density unit
SC	Stomatal conductance
RCBD	Randomized complete block design
ROS	Reactive oxygen species
v/v	Volume per volume
nm	Nanometer
A_{663}	Value of absorption at wavelength 663 nm
A_{645}	Value of absorption at wavelength 645 nm
Chl <i>a</i>	Chlorophyll <i>a</i>
Chl <i>b</i>	Chlorophyll <i>b</i>
μl	Microlieter
FFA ₇₀	Preservative (1 Formalin: 1 Acetic acid: 17 70% Ethanol alcohol)
Na_2CO_3	Sodium carbonate
mM	Millimol
ANOVA	Analysis of variance

CHAPTER 1

INTRODUCTION

Salinization is a major abiotic stress factors considered to be the main source of the delayed germination, yield reduction and limiting plant development. The salinity of soil and water is caused by soluble salts due to the deterioration and dissolving of rock, as well as concentrated as a result of evaporation. Plants are divided into two groups based on the difference in their ability to tolerate high salt concentrations; Glycophytes are highly susceptible to salinity and tolerate low concentration of salts whereas halophytes can grow and survive in saline environments and tolerate moderate-to-high concentration of salts (Munns and Tester 2008). Most crops, for example, *Corchorus olitorius*, belong to glycophyte group which gets affected by salinity due to which yield reduction and development issue rises. Furthermore, halophytes show signs of reduction when salinity reaches a certain threshold and become toxic (Shabala, 2013).

Corchorus olitorius is herbal plant, belong to glycophytes group which is popularly known as Molokhia in Middle East and highly consumed especially in Egypt, Palestine, and Jordan. It is an essential green, leafy edible vegetable that is mainly known for its fiber product, jute, iron, vitamins A, C, K and folate (Imbamba, 1973). Salinity associated with low soil moisture causes salt stress which affect seed germination, seedling growth and crop establishment.

The salinity of soil and irrigation water one of the greatest environmental strains affecting plant growth and development. Salinity influences agricultural production in many parts of the world (Shahbaz *et al.*, 2012; Taneenah *et al.*, 2015). The increase in the salinity of soil and water lead to the reduction of crop production, thereby adding to the continuing impact on the validity of agricultural land, and turning it into the marginal land (Paul, 2012). At least 20% all of irrigated lands and 50% of cropland have been detected to be under the effect of salt stress (Pitman and Lauchli, 2002; Machado and Serralheiro, 2017). Continuous using of saline irrigation water intensifies the problem of salinity and leads to soil degradation which will become more acute in the future (Yan *et al.*, 2015; Machado and Serralheiro, 2017); hence, threaten the food security worldwide.

At present, salinity stress extent in all climatic regions also found in places below sea level, such as the area around the Dead Sea and in high mountainous regions (Pitman and Läuchli, 2002; Manchanda and Garg, 2008). Dead Sea water is the lowest spot on earth, which is located in the Middle East, between Jordan and Palestine. Dead Sea water is 10 times saltier than the ocean water. Salts carried into the river going to the Dead

Sea will not escape the sea, salts are kept and accumulated through water evaporation. In many developing countries, there are extensive coastal lands where sea water and harvested water were utilized for crop production. These water resources are usually neglected, and can be used for growing halophytes (Radulovich *et al.*, 2017). The greatest advantage of seawater agriculture is that it opens up coastal lands, which were previously unusable for agricultural production due to the fresh water shortage (Magbol *et al.*, 1996). Until to date, there is no comparison study had been conducted on the effect different salinity sources on the seed germination of *Molokhia*. Therefore, this study aimed using three different sources of salinity which are Dead Sea water, sea water and NaCl on the germination, anatomical, and growth and antioxidant of *Molokhia*.

Objectives of study are:

1. To study the effect of the Dead Sea water, sea water, and NaCl on the germination of *Molokhia* seeds.
2. To study the effect of the Dead Sea water, sea water, and NaCl salinity on the growth, stomatal conductance, and anatomical leaves of *Molokhia* seedlings.
3. To study the antioxidant profiling of *Molokhia* leaves as response to Dead Sea water, sea water and NaCl.

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