

## Salinity Effect on the Germination and Some Chemical Components of *Matricaria* spp

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*Chamomilla recutita* (L.) (Syn: *Matricaria recutita* and *Matricaria chamomilla*), is the most important medicinal plants. An objective of this study is to investigate the effects of salinity levels on yield and yield components in germination and growth stage of two chamomile species. The experiment completed in 2006 in the agriculture college laboratory of Shahrekord University and Isfahan Agriculture Research Center. Plants were grown in the agronomy laboratory of Shahrekord University for germination test. The treatments included salt levels of control, 6, 12 and 18 dSm<sup>-1</sup>. The genotypes were cultivated at greenhouse in sand irrigated with nutrient solutions. The salinity stress in this trial exerted at 2 durations. The first from seedlings stage (35 days after emergence with plants at 8-10 leaves) until the end of the experiment (about 3 months). The second duration of stress exertion began at stem elongation and seedlings emergence from rosette stage to harvest (about 1.5 month). This experiment carried out as a split split plot with 3 replication on the based of CRD design. The traits measured stem and root dry matter yield with stem relative water, root relative water and Na, K, and Ca content in relation to salinity effects on seedling stage and stem formation. The salt application trials indicated that dry matter yield was decreased with increasing NaCl doses. Effect of salt treatment on genotypes was decreased with increasing salt concentration in all treatments, but Na increased with increasing salt concentration either stem or root. All the criteria investigated suggest therefore that *M. aurea* were superior to *M. recutita* genotypes. Positive and highly significant relationships existed between stem dry matter yield and all its components with the exception of the stem relative water ( $r = 0.053$ ). But these significance were negative in Na stem (-0.309\*\*) and Na root (-0.170\*\*). There were also highly positive significant relationship between root dry matter yield and all its examined characters with exception of Na stem (-0.487\*\*) and Na root (-0.218\*\*). There was a significant difference between genotypes studied for all traits except for root relative water content. The *Matricaria aurea* genotypes revealed more tolerance to against salinity specially Isfahan and Mashhad genotypes.

**Key Words:** *Matricaria recutita*, *Matricaria aurea* L, Salinity, Germination, Relative water.

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## INTRODUCTION

Chamomile, *Chamomilla recutita* (L.) Rauschert, is the most favored and most used medicinal plants. Phytotherapeutically useful are primarily flower anthodia, and this drug (*Flos chamomillae*) is included into the pharmacopoeias of 26 countries all over the world<sup>1</sup>.

A long chamomile history and genesis of chamomile plant use springs from its origin. The native world area of chamomile plant species was a Frontal Asia. One from eight of the Gene Pool Centers is mainly Iran and this area gave a base for a cultivation and domestication of grains and probably chamomile species, as a weed, too. Chamomile can be found in the secondary plant communities, such as trodden societies on dry and moist soils, weed societies and dump societies<sup>2</sup>.

Seed germination and early seedling growth are critical stages for the establishment of plant populations under saline conditions<sup>3</sup>. Grasses differ in their upper limit of salinity tolerance and an increase in salinity concentration usually delays and reduces seed germination<sup>4,5</sup>. Seed germination under saline conditions occurs after high precipitation where soil salinity is usually reduced due to leaching.

One study about salinity effect on chamomile showed that germination amount, germination rate, radicle and shoot length reduced significantly by increasing salinity levels. Comparing the two species for the evaluated traits shown, that *M. aurea* was better for the germination amount, germination rate, shoot length, however *M. chamomilla* was better for radicle length. Over all, the Isfahan accession *M. aurea* show the best performant for the traits except for radicle length and the Italy *M. chamomilla* accession had the less amounts of the traits at different levels of salinity<sup>6</sup>.

Tolerance to abiotic stresses is associated with modifications of morphological and physiological traits. These include changes in plant architecture, variation in leaf cuticle thickness, stomatal regulation, germination, antioxidant capacity, hormonal regulation, membrane and protein stability, maintenance of photosynthesis and root morphology<sup>7</sup>. Soil salinity is one of the most significant abiotic stresses for crop plants, including legumes<sup>8,9</sup>. These latter plants are very important both ecologically and agriculturally because legume roots are able to interact symbiotically with soil microorganisms to form nodules that fix atmospheric nitrogen. Hence, legumes are interesting candidates for improving soil fertility and incorporating salty soils into agriculture. In general, high NaCl concentrations produce water deficit, ion toxicity, nutrient imbalance and oxidative stress<sup>10</sup>. These adverse effects cause modifications of root morphology and inhibition of plant growth and can result in plant death. In Arabidopsis, alterations in root morphology caused by external environmental conditions are perturbed in several hormone related mutants, suggesting a connection between perception of soil conditions and modification of endogenous phytohormonal balances to establish new root architecture<sup>11,12</sup>. In legumes, the root lateral organs, nodules and lateral root are also regulated by diverse hormonal, metabolic and environmental signals<sup>13</sup>. It is well documented for abiyotik stress that a coordinated crosstalk amongst drought, cold and high salinity pathways exist<sup>14-17</sup>.

There is not much information has been reported on seeding densities in chamomile under salinity. Our objectives are to investigate the effects of salinity levels on grain yield and yield components, analyze the relationships of the yield components and determine relative water, Na, K and Ca content in stem and roots. Yield loss under salinity would be compensated for by increasing seeding density above normal density levels.

## EXPERIMENTAL

Eight accessions of chamomile seed collected from natural site of Iran. Four accessions of *M. recutita* belong to Isfahan, Zabol (parts of Iran) and Hungry and Italy and four accretions of *M. aurea* belong to Isfahan, Tabriz, Shahrekord and Mashhad (different parts of Iran).

**Germination test:** This experiment was conducted to investigate salinity effects on two spices of chamomile (*M. recutita* and *M. aurea*) during germination and early seedling growth at this experiment again 4 salinity levels (control, 6, 12, 18 ds/m).

Evaluation for salinity tolerance during germination was accomplished by placing 50-seed samples in 90 by 15 mm petri dishes containing one blotter paper to which 5 mL of distilled water or various solutions of NaCl were added. Germination responses to concentrations of control (0), 6, 12 and 18 dSm<sup>-1</sup> of the combined salts were determined. Electrical conductivities were measured with a Model metrohm 644 conductivity bridge (The Co., Swiss.). The covered petri dishes were arranged in an incubator in a completely randomized experimental design. Germinated seeds counts were made at 3, 5, 7 and 19 days after initiation of germination (DAI). Analyses of variance and orthogonal contrasts were used to analyze the data (SAS Institute 1995). Data for each counting date were analyzed independently. A factorial experimental base on completely randomized design with three replications was used at the experiments. The treats consisting: germination amount, speed germination, Stemlet and radicle length.

**Speed of the germination:** For determination of speed germination we count germinated seed on 3, 5, 7, 10, 14 and 19 days after germination and used this formula:

$$SG = \Sigma(N_i/D_i)$$

SG is the speed germination and  $N_i$  is the number of seed germinated and  $D_i$  is the day after germination<sup>18</sup>.

Germination no is the number of germinated seed in each petri used ratio for determination amount because one can't use % germination for growth condition, seed vigor, seed mature and harvest condition could be different for each genotype. Seed of genotypes can be different vigor for germination in same condition (control or each salinity levels), Ratio for each salinity level for each genotype counted with germinated seed divided for germinated seed of control level.

**Plant culture and salinity treatments and growth conditions:** The experiment were conducted in the green house at Isfahan, Iran (32°40' N latitude and 51°52' E longitude) between August 2005 to January 2006. The plants were cultured in plastic plot (40 cm × 60 cm × 20 cm deep) filled with sand and irrigated with nutrient solution. Seed were planted with one row per genotype and 6 genotype of plot. The rows were spaced 10 cm apart with 20-30 seeds per row sowing depth less than 1 cm, days after sowing plants were thin and 3-5 plants remain in each row. Air temperature ranged from 23 to 26 °C during day and 12 to 15 °C during the night.

The experiment in controlled environment examined the effects of two duration and four salinity levels on the chamomile plant. The four salinity levels were including control, 6, 12 and 18 dSm<sup>-1</sup> that applied at the both salinity duration. The first salinity was started at seedling stage, when the plants height was about 6-7 cm and the second duration consisted from the stem formation. The both salinity duration were dispense separate and continued to the end of growth stage. This experiment operated as a split split plots with three replications on the base of the CRD design. The traits measured in this experiment included stem dry weight, root dry weight, stem relative water, root relative water, Na, K and Ca content of stem and root part of plants.

**Determination of dry weight of plant stems and roots:** Dry weight of plant stems and roots were measured at 30 and 45 days intervals after the highest salt concentration was reached. Dry mass was determined after drying for 48 h in a forced-draft oven at 75 °C.

**Determination of relative water content in stem and root:** Water content of root and stem tissue was calculated on a tissue dry weight basis, *i.e.* gram water per gram dry weight of tissues by the following formula<sup>19</sup>:  $\text{Water} = \frac{\text{F.wt} - \text{D.wt}}{\text{D.wt}}$  where, F.wt.= fresh weight of tissue and D.wt.= dry weight of tissue.

Plant samples were oven dried (75 °C to constant mass for 48 h) and weighed. Shoot samples were ashes at 550 °C for 3 h. Inorganic ions were then extracted well ground shoot samples of 200 mg were digested in 10 mL of HCl. After digestion, the volume of each sample was made up to 100 mL. Na, K and Ca were determined with a flame photometer (Jenway, PFP-7).

The data were analyzed using general linear models with SAS (version 6.12) and the procedures were described by SAS (SAS Inst., 1994). The measurements of treatments were compared and grouped using Duncan's multiple range tests at the 0.01 significance level. The comparisons were made only between the lowest salt or seeding density levels and the other treatment levels. The relative importance of yield components was analyzed using multiple factorial analyses.

## RESULTS AND DISCUSSION

Dry matter yield varied significantly depending on treatments and period of salt application ( $p < 0.01$ ). Effect of salt treatment on dry matter yield was higher in seedling stage (0.34 g plant<sup>-1</sup>) than stem formation (0.42 g plant<sup>-1</sup>) this can be seen

on root dry matter, Ca in stem, Ca/Na stem, and Ca in root. On the other hand remain treatment had opposite effect with salt application. Effect of salt treatment on genotypes was decreased with increasing salt concentration (Table-3) in all treatments but Na increased with increasing salt concentration either stem or root.

Salt application period was highly significant for all the characters evaluated, except for the root relative water, Na in stem, K/Na in stem, and Ca/Na in stem. All of the examined characters were significantly affected by salinity. The period x salinity interaction was highly significant for RDM, Na in stem; on the other hand SDM, SRW and Ca in root were not significant. The genotypes effect was highly significant almost all characters except for the RRW. Period X genotype interaction was highly significant for SDM, RDM, Na, Ka and Ca both in stem and in root extraction. There were highly significant relationships examined with salinity X genotype interaction and also the period X salinity X genotypes interactions with exception of RRW, K in stem, and Ca/Na in stem (Table-4).

ANOVA mean squares for salinity effect on seed germination were highly significant for *M. recutita* and *M. aurea* in relation to all stem and root formation characters examined (Table-2). All the criteria investigated suggest therefore that *M. aurea* were superior to *M. recutita* genotypes. The highest stem measured 9.6 mm in Shahrecord genotypes after 21 days of germination. Measurement of root indicated that Zahol genotypes had the longest root developed plants. During the germination period Hungary ecotypes had the lowest speed (2.5). On the other hand, Tabriz genotypes had the highest speed (25.8) of germination (Table-1). There was a serious concern that plant stand and the development of yield components were affected by water salinity<sup>7,20</sup>. Soil salinity is one of the most significant abiyotik stresses for crop plants, including legumes<sup>8</sup>. In general, high NaCl concentrations produce water deficit, ion toxicity, nutrient imbalance and oxidative stress<sup>10</sup>. These adverse effects cause modifications of root morphology and inhibition of plant growth and can result

TABLE-1  
SALINITY EFFECT OF SEED GERMINATION IN RELATION TO  
*M. recutita* AND *M. aurea* GENOTYPES

Genotype	Stem	Root	Speed	Ratio	Root/Stem	Ger. No.
<b><i>M. recutita</i></b>						
Hungary	2.8c	1.8d	2.5g	0.56b	0.62c	3.4c
Zabol	4.9b	17.9a	17.2c	0.57b	3.1a	7.3b
Isfahan	5.1b	10.6b	4.8f	0.48bc	1.3bc	3.5c
Italy	5.2b	10.9b	14.7d	0.57b	1.9b	6.6b
<b><i>M. aurea</i></b>						
Mashad	5.1b	11.2b	10.6e	0.45c	1.5b	6.9b
Isfahan	6.0b	10.9b	23.6b	0.74a	1.6b	11.2a
Tabriz	8.9a	5.8c	25.8a	0.83a	0.68c	10.2a
Shahrekord	9.6a	5.5c	17.4c	0.54bc	0.62c	4.3c
Mean	5.31	9.33	14.58	0.59	1.25	6.68

\*The differences between the values with the same letters are insignificant at  $p < 0.01$ .

TABLE-2  
ANOVA MEAN SQUARES FOR SALINITY EFFECT ON SEED GERMINATION FOR  
*M. recutita* AND *M. aurea* IN RELATION TO STEM AND ROOT FORMATION

Source	df	Stem	Root	Speed	Ratio	Root/Stem	Ger. No.
Genotype	7	60.9**	287.3**	822.3**	0.19**	9.0**	102.5**
Salinity	3	221.8**	954.1**	2510.5**	2.9**	5.9**	144.8**
Gen.*	21	7.2**	116.1**	113.6**	8.9**	1.4**	11.8ns
Error	64	2.05	15.2	4.14	1.62	0.63	7.14
Total	95						

TABLE-3  
STEM AND ROOT DRY MATTER YIELD WITH STEM RELATIVE WATER,  
ROOT RELATIVE WATER AND Na, K AND Ca CONTENT IN *M. recutita*  
AND *M. aurea* IN RELATION TO SALINITY EFFECTS ON SEEDLING  
STAGE AND STEM FORMATION (g plant<sup>-1</sup>)

Treatment	Check	6 dSm <sup>-1</sup>	12 dSm <sup>-1</sup>	18 dSm <sup>-1</sup>	Seedling stage	Stem formation	Mean
SDM	0.57	0.40	0.30	0.26	0.34	0.42	0.38
RDM	0.23	0.15	0.12	0.10	0.10	0.20	0.15
SRW	79.27	75.82	72.78	69.42	76.60	72.04	74.32
RRW	83.59	80.74	76.92	76.54	79.17	79.73	79.45
Na stem	5.48	11.40	14.93	17.85	13.49	11.35	12.42
K stem	25.24	18.91	13.73	16.07	20.90	16.08	18.49
K/Na stem	7.29	2.07	1.10	1.16	3.10	2.71	2.90
Ca stem	15.64	11.43	10.37	9.40	10.11	13.32	11.71
Ca/ Na stem	4.14	1.15	0.86	0.62	1.41	1.98	1.69
Na root	5.35	10.17	13.10	17.48	13.30	10.20	11.75
K root	7.52	5.87	5.20	5.09	6.58	5.26	5.92
K/Na root	1.78	0.52	0.33	0.24	0.81	0.63	0.72
Ca root	21.19	17.42	14.11	12.35	13.99	18.54	16.27

in plant death. It is well documented for abiyotik stress that a coordinated crosstalk amongst drought, cold and high salinity pathways exists<sup>17,21,22</sup>.

Positive and highly significant relationships existed between SDM yield and all its components with the exception of the stem relative water ( $r = 0.053$ ). On the other hand this significance were negative in Na stem ( $-0.309^{**}$ ) and Na root ( $-0.170^{**}$ ). There were also highly positive significant relationship between RDM and all examined characters with exception of Na stem ( $-0.487^{**}$ ) and Na root ( $-0.218^{**}$ ). In general, the components had significant positive and negative correlation with each other (Table-5). Comparing wild plants with cultivated forage crops, wild crops may have had some advantages. Some wild plants are more resistant to negative environmental condition (salinity, drought and cold resistant) diseases and pest damages<sup>23</sup>. Total biomass yield increased when plant size (as stem yield) would increased<sup>24</sup> ( $r = 0.581^{**}$ ). Forage yield positively related with stem yield m<sup>-2</sup> ( $r = 0.920^{**}$ ) it could be possible to develop with high forage yielding population and also produce substantial amount of high quality yields on sweet brome grass. Simple

TABLE-5  
SIMPLE CORRELATION COEFFICIENT OF STEM AND ROOT DRY MATTER YIELD WITH Na, K AND Ca  
COMPONENTS IN *M. recutita* AND *M. aurea* GENOTYPES

	Stem dry weight (g)	Root dry weight (g)	Stem relative water	Root relative water	Na-stem	K-stem	K/Na stem	Na-root	K-root	K/Na-root	Ca-stem	Ca/Na-stem
SDW	-											
RDW	0.325**	-										
SRW	0.053ns	0.191**	-									
RRW	0.233**	0.179*	0.375**	-								
Na -S	-0.309**	-0.487**	-0.267**	-0.186**	-							
K -S	0.201**	0.230**	0.513**	0.309**	-0.385**	-						
K/Na -S	0.232**	0.457**	0.320**	0.119ns	-0.588**	0.593**	-					
Na -R	-0.170*	-0.218**	0.120ns	-0.126ns	0.378**	0.022ns	-0.257**	-				
K- R	0.161*	0.275**	0.388**	0.153*	-0.224**	0.533**	0.479**	0.323**	-			
K/Na - R	0.087ns	0.306**	0.234**	0.130ns	-0.310**	0.478**	0.643**	-0.231**	0.690**	-		
Ca -S	0.260**	0.359**	-0.107ns	0.037ns	-0.235**	0.024ns	0.233**	-0.533**	-0.029ns	0.351**	-	
Ca/Na- S	0.219**	0.510**	0.164*	0.071ns	-0.548**	0.320**	0.873**	-0.378**	0.323**	0.582**	0.499**	-
Ca -Root	0.213**	0.329**	-0.080ns	-0.005ns	-0.392**	-0.050ns	0.255**	-0.583**	-0.297**	0.066ns	0.589**	0.394**

TABLE-4  
MEANS AND ANOVA MEAN SQUARES FOR STEM AND ROOT DRY MATTER YIELD PER PLANT AND  
ITS COMPONENTS IN FACTORIAL ANALYSIS

Source of variation	df	SDM	RDM	SRW	RRW	Na stem	K stem	K/Na stem	Ca stem	Ca/Na stem	Na root	K root	K/Na root	Ca root
Mean		0.38	0.15	74.3	79.5	12.42	18.19	2.90	11.71	1.69	11.19	6.35	0.95	16.27
Period	1	0.32**	0.514**	101.0*	15.1 ns	219.8ns	915.5	7.2ns	494.0**	15.5ns	426.7**	71.7*	2.2	993.1*
Error (a)	2	0.001	0.001	23.9	4.7	12.5	10.7	5.3	4.1	3.0	2.8	0.8	3.3	4.9
Salinity	3	0.93**	0.163**	850.9*	538.6*	360.0**	988.6**	420.0**	362.4**	129.8**	829.6**	38.7**	56.8**	729.0**
Error (b)	6	0.04	0.000	102.8	72.8	54.1	33.1	2.7	0.7	1.3	51.4	3.5	0.01	5.8
Period x Salinity	3	0.03ns	0.027**	314.0 ns	382.4*	113.2**	250.7**	7.1	23.6*	0.7	134.0*	5.7	1.3	18.9ns
Error (c)	6	0.01	0.001	67.2	57.1	5.1	15.2	8.6	3.8	2.6	14.3	6.3	3.6	4.5
Genotypes	7	0.42**	0.079**	796.6**	130.4 ns	104.5**	689.6**	70.9**	137.5**	8.6**	282.6**	455.2**	42.3**	450.2**
Period X Gen.	7	0.38**	0.013**	65.2*	127.8 ns	87.0**	19.6**	4.2ns	14.0**	3.2ns	243.1**	50.3**	0.5	46.9**
Salinity X Gen.	21	0.05**	0.010**	131.2**	111.7*	77.4**	162.8**	35.5**	44.7**	9.3**	53.5**	100.9**	33.3**	30.2**
Per. X Sal. X Gen.	21	0.06**	0.004**	55.0*	84.9 ns	52.1**	33.3	1.7	13.4**	2.3ns	65.7**	28.1**	1.9	32.1**
Error (d)	112	0.04	0.001	30.2	63.2	13.2	28.7	3.7	1.8	2.0	14.9	7.2	2.1	5.3

\*Na, K and Ca were measured mg g<sup>-1</sup>

coefficient analysis has been used successfully to determine the various plant characters on yield and yield components *Triticum aestivum*<sup>25</sup>, in *Cicer arietinum* L<sup>26</sup>, in *Medicago sativa* L.<sup>27</sup>.

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