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Studying the Effects of Salinity, Aridity and Grazing Stress on the Growth of Various Halophytic Plant Species (*Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans*)

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Abstract: Determining plant resistance to salinity, aridity and grazing is very important for selecting the favorable plant species for rangelands. The objective of this study was to compare the salinity, aridity and grazing tolerance of three rangeland species (*Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans*). The experiment was conducted in a greenhouse, with a factorial arrangement in a completely randomized design using 3 replications. Salinity levels of 4 (as control), 20, 30 and 40 dS/m; Aridity levels of field capacity irrigation (as control), -12 and -14 bars; Grazing levels of 0% (as control), 25%, 50% and 75% cutting were applied. The results showed that increasing salinity and aridity level caused reduction in dry matter production and residual dry matter in all studied plant species. 25% cutting caused increment in residual dry matter. Increasing grazing level caused increment in dry matter production. The maximum survival scores were found in the lowest salinity, aridity and grazing level treatments. However, the minimum survival scores were observed in the highest salinity, aridity and grazing level treatments. *Agropyron elongatum* had the maximum and *Kochia prostrata* had the minimum salinity, aridity and grazing tolerance.

Key words: Dry matter production · Residual dry matter · Survival scores

INTRODUCTION

Soils with electrical conductivity (EC) more than 2 dS/m are considered saline [1]. The plants that can be naturally established in saline soils called halophytes [2]. The plants that can be naturally established in drought soils called xerophytes [2]. Salinity, aridity and grazing stress causes reduced in nutrient uptake by roots and eventually plant death [3]. Thus, soil salinity; soil aridity and grazing stress can reduce rangeland production potential [4]. Salt-affected lands that are covered with halophytic plant species and provide forage for livestock grazing are called saline rangelands [6]. Extreme grazing of saline and arid rangelands causes more destruction to these areas [5]. Therefore, saline and arid rangeland management and species selection is needed for providing forage for the livestock [5, 7]. Aridity is one of the most important factors influencing this area's vegetation survival. Only well-adapted species are present [8]. The climate of this arid to semiarid region is defined by generally low and highly variable precipitation [9]. The average precipitation of Incheh Boroun rangelands is 240 mm/year [9]. Incheh Boroun Rangelands is located between 54° 63' 20" to 54° 97' 43" eastern longitude and 36° 92' 62" to 37° 32' 42" northern latitude in Golestan Province. Golestan is a northern Iranian Province that locates in northern east of Iran. Vegetation management is the most important factor in saline and arid lands management [5]. A significant portion of Iran's land is considered saline and arid rangeland [8]. A vast part of the Golestan Province rangeland is affected by salinity and aridity [10]. Adaptation to the high salinity and aridity condition has been seen only in a few native plant species in Incheh Boroun rangeland and the rangeland vegetation of this area is very poor [10]. Thus, the forage production of plant species in Incheh Boroun is not sufficient for the livestock needs [10]. Identifications and introductions of the halophytic plant species that can be adapted to the salinity, aridity and grazing stress conditions of this region are essential for increasing vegetation production in this area.

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Therefore, according to salinity and grazing resistance, drought tolerance and palatability of the species, three halophyte plant species (*Agropyron elongatum, Kochia prostrata* and *Puccinellia distans*) have been selected in this study for the Incheh Boroun rangeland improvement.

There are several studies found on the effects of salinity, aridity and grazing stress on the growth of halophytic plant species [12, 16-18].

Salinity tolerance of three rangeland grasses in the greenhouse condition was investigated [13]. Their results showed that the dry weights of the roots and the shoots in *Agropyron elongatom* were more than that of *Agropyron desertorum* and *Hordeum fragilis.* Adaptation (establishment and survival) of some rangeland plants of Chapar Ghoymeh saline rangeland was investigated [14]. The results of this research [14] showed that *Atriplex canescens, Artemisia sieberi* and *Agropyron elongatum*had better establishments and higher survival scores than other studied plant species.

Resistant to salinity, shoot height and root length of *Agropyron desertorum* was more than that of *Agropyron elongatom* [15]. The results of the studies [18, 19] showed that *Agropyron elongatom* had high resistance to soil salinity. Soil salinity caused reduction in shoot height, shoot dry weight, shoot fresh weight, root dry weight and root fresh weight at various stages of phenology in *Agropyron elongatom* [20]. The effect of soil salinity on *Puccinellia distans* was studied [11]. The results of this research [11] showed a significant reduction in shoot dry weight and shoot fresh weight. The adverse effect of salinity on shoots was reported more than that on the roots in *Puccinellia distans* [21].

Drought, like many other environmental stresses, has adverse effects on dry matter production and plant residual dry matter. Drought is the most limiting factor in vegetation production [22-24]. *Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans* are reported to be relatively tolerant to water stress [22-25].

Plant responses to salt stress and water deficit have much in common. Salinity reduces the ability of plants to take up water and this causes reductions in growth rate along with a suite of metabolic changes similar to those caused by water stress. Hence, the ability of a plant to grow under these environmental stress conditions is a key factor to improve rangeland vegetation in saline and arid rangelands [22-25]. Water deficit alters plant growth rate and nutrient uptake [22-25].

Residual plant dry matter accumulation decreased significantly by water stress [25]. Dry matter production can vary with the degree of water stress [24].

Grazing intensity is a major determinant of forage growth and productivity. Research findings on these effects are not always conclusive. Some researchers found no significant effects of grazing on forage re-growth potential but others observed reduction in forage re-growth under intensive grazing. The extrapolation of field observations is often limited because of their location and time specificity. On the other hand, models synthesize knowledge of the systems behavior so that once validated, they provide effective tools for investigating livestock and forage growth under a wide range of environmental and management conditions [26].

The objectives of this study were to compare and evaluate the growth responses of 3 halophytic plant species (*Agropyron elongatum, Kochia prostrata and Puccinellia distans*) under salt, drought and grazing stress conditions.

MATERIALS AND METHODS

The seeds of *Agropyron elongatum* and *Puccinellia distans* were taken from around Aqala City. *Kochia prostrata* seeds were prepared from Isfahan Agriculture and Natural Resources Research Center.

Plants were grown in Gorgan University of Agricultural Sciences and Natural Resources greenhouse at 15-40°C under a photoperiod of 16 h. *Agropyron elongatum, Kochia prostrata and Puccinellia distans* seeds were planted in pots of 14 cm diameter and 25 cm depth; each pot contained 3.5 kg soil that prepared from Incheh Boroun area. The soil characteristics were as follows:

Entisol in type, sandy clay loam in texture, sand 53.7%; silt 7.14%; clay 39.16%; pH 9.63 and organic matter 0.87%.

For the seed germination test in the laboratory, 100 seeds were planted in 4 Petri dishes on the moistened germination papers. Then, the 4 Petri dishes were placed in a germinator under alternating 20-30°C temperature. An initial seed germination count was made after 4 days and a final count after 7 days [22]. Twenty [15] seeds of each species were planted in each pot and five replicates were used for each treatment [12].

The average soil surface salinity of Incheh Boroun is 28 dS/m [9]. Therefore, salinity levels of 20, 30 and 40 dS/m as different levels of salinity treatments and salinity level of 4 dS/m as control were applied on the pots. Sodium chloride (NaCl) was used to make salinity treatments. Adds 0.64 gr of NaCl per one soil Kg increased one grade salinity level (in term of dS/m). Pots that used in this study are 3.5 Kg. So weight of salt

needed at each salinity level was calculated [27]. The salinity treatments at 4 levels [4 (control), 20, 30 and 40 dS/m] were made with this method [12].

Different levels of simulated grazing have been conducted on the pots to determine the resistance of plant species to grazing. Dry matter produced and residual dry matter in each grazing level was compared with dry matter production in the control level. The plants in control pots were not clipped with 25%, 50% and 75% clipping applied as simulated grazing levels [28].

3 levels of aridity levels have been implemented on pots with Pressure plates. Aridity levels of field capacity irrigation (as control), -12 and -14 bars were applied. Each pot was weighed every 3 days. The decreased weight of each pot in each round of review showed the amount of water evaporated or consumed by the plants. So this weight of water is added pots by irrigation water [1]. Dry matter production and residual dry matter under aridity stress were calculated in this stage of study.

The variables and the levels of the salinity treatments based on factorial arrangement in a completely randomized design for the pot culture are shown in Table 1.

At the end of the phenology stage of the halophytic plant species, the average of dry matter production and residual dry matter in each pot was measured in gr. The pot soil was rinsed with distilled water. The residual dry weight was measured after oven drying the plants at 60°C for 48 hours.

The survival scores were recorded weekly by scoring in the scales of 0 to 4 (0 score for the dried plant and 4 for the very vigorous plant) during the greenhouse experiment [13].

Dry matter production (gr), residual dry matter (gr) and survival scores data were standardized to compare the effect of salinity on *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans.*

Equation 1 was used to standardize the data of this research.

$$\text{Equation 1 } St = \frac{N}{N\max}$$

S_t : Standardized data of studied treatments
N : Data in each replicate
N max : Maximum data of all replicates (29).

After the data standardization, ANOVA was performed for statistical analysis of the data. Statistical significance was considered at $P < 0.05$.

Table 1: Variables and the levels of treatments based on factorial arrangement in a completely randomized design

Variables	Variable Numbers	Variable Names
	3	*Agropyron elongatum*
Species		*Kochia prostrata*
		Puccinellia distans
		Fc irrigation
Aridity (bar)	3	-8 bars
		-12 bars
		EC = 4 (Control)
Salinity (dS/m)	4	EC= 20
		EC= 30
		EC= 40
		0% cutting (control)
	4	25% cutting
Grazing (cut percentage)		50% cutting
		75% cutting
Replications	5	-----

Results

Dry matter production and residual dry matter at all stages of development were reduced progressively with increasing salinity concentrations. Relative percentage of dry matter production and residual dry matter of salinized, water stressed and simulated grazed plants compared to those of the controls were computed as (salinized, water stressed and simulated grazed on plants/control plants) x100 and illustrated in Figures 1, 2 and 3.

Results of the analysis of variance (ANOVA) of the main effects and interactions effects of different species, salinity, aridity and grazing treatments on dry matter production and residual dry matter are shown in table 2 ($p < 0.05$).

Means comparisons of the species types effects on dry matter production and residual dry matter is showed in Table 3.

Increased salt concentration in the soil significantly decreased ($p < 0.05$) dry matter production and residual dry matter (Table 4).

Soil aridity level increment due to significantly decrease in ($p < 0.05$) dry matter production and residual dry matter (Table 5).

Results of the analysis of variance (ANOVA) of effects of different grazing treatments on dry matter production and residual dry matter are shown in Table 6 ($p < 0.05$).

Results of the analysis of variance (ANOVA) of interaction effects of salinity×aridity treatments on dry matter production and residual dry matter are shown in Table 7 ($p < 0.05$).

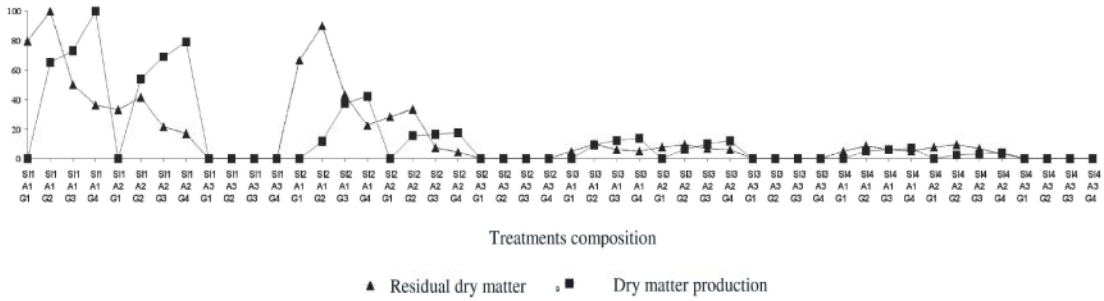

Fig. 1: Relative percentage of dry matter production and residual dry matter of salinized, water stressed and simulated grazed plants in *Agropyron elongatum* compared to those of the controls.

S: Species abbreviation *(Agropyron elongatum)*

Sl: Salinity levels. Sl1, Sl2, Sl3 and Sl4 are abbreviation of 0, 20, 30 and 40 dS/m for salinity levels

A: Aridity level. A1, a2 and A3 are abbreviation of Fc, -8 and -12 bars for aridity levels

G: Grazing. G0, G1, G2 and G3 are abbreviation of 0, 25, 50 and 75% cutting for grazing levels

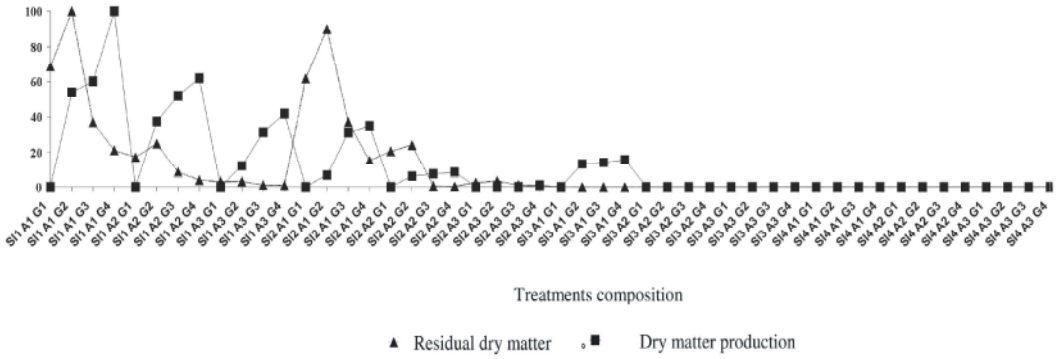

Fig. 2: Relative percentage of dry matter production and residual dry matter of salinized, water stressed and simulated grazed plants in *Kochia prostrata* compared to those of the controls.

S: Species abbreviation *(kochia prostrata)*

Sl: Salinity levels. Sl1, Sl2, Sl3 and Sl4 are abbreviation of 0, 20, 30 and 40 dS/m for salinity levels

A: Aridity level. A1, a2 and A3 are abbreviation of Fc, -8 and -12 bars for aridity levels

G: Grazing. G0, G1, G2 and G3 are abbreviation of 0, 25, 50 and 75% cutting for grazing levels

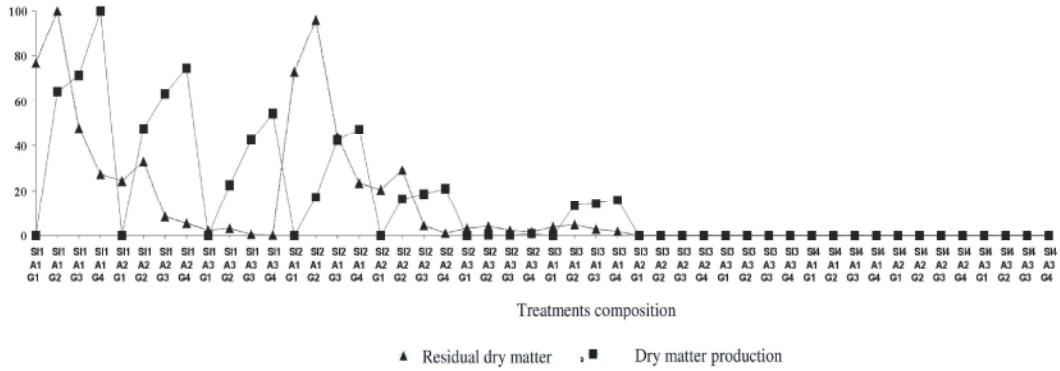

Fig. 3: Relative percentage of dry matter production and residual dry matter of salinized, water stressed and simulated grazed plants in *Puccinellia distans* compared to those of the controls.

S: Species abbreviation *(Puccinellia distans)*

Sl: Salinity levels. Sl1, Sl2, Sl3 and Sl4 are abbreviation of 0, 20, 30 and 40 dS/m for salinity levels

A: Aridity level. A1, a2 and A3 are abbreviation of Fc, -8 and -12 bars for aridity levels

G: Grazing. G0, G1, G2 and G3 are abbreviation of 0, 25, 50 and 75% cutting for grazing levels

Table 2: Results of the analysis of variance (ANOVA) of the main effects and interactions effects of different species, salinity, aridity and grazing treatments on dry matter production and residual dry matter

		Main effects and interactions effects on dry matter production			Main effects and interactions effects on residual dry matter		
Sources of Variations	df	MS	F	P	MS	F	P
Species	2	0/53	521/45	*<0/00	1/04	231/32	*<0/00
Salinity Level	3	2/40	2351/06	*<0/00	3/28	1157/11	*<0/00
Aridity level	2	0/77	755/13	*<0/00	1/20	351/02	*<0/00
Grazing level	3	1/38	1358/22	*<0/00	0/94	1125/39	*<0/00
Species×Salinity	6	0/17	168/24	ns 0/05	0/43	127/37	ns 0/13
Species×Aridity	4	0/02	26/04	ns 0/06	0/46	11/09	ns 0/06
Species×Grazing	6	0/09	97/19	ns 0/05	0/12	83/12	ns 0/04
Salinity×Aridity	6	0/16	161/32	*<0/00	0/23	129/15	*<0/00
Salinity×Grazing	9	0/4	424/62	ns 0/06	0/17	278/29	ns 0/06
Aridity×Grazing	6	0/09	94/55	ns 0/05	0/07	83/09	ns 0/08
Species×Aridity× Grazing×Salinity	96	0/0	17/81	ns 0/06	0/03	13/84	ns 0/07

* significant at 0/05 MS: Mean of Squares F: Fisher Statistics
ns: not significant at 0/05 df: degree of freedome P: significant Level

Table 3: Means comparisons of the species types effects on dry matter production and residual dry matter

	Species type effect on dry matter production	Species type effect on residual dry matter
Species type	Mean	Mean
Agropyron elongatum	0/36 a	0/39 a
Puccinellia distans	0/27 a	0/34 a
Kochia prostrata	0/19 b	0/ 14b

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level

Table 4: Means comparison of different levels of salinity treatments on dry matter production and residual dry matter

	Salinity effect on dry matter production	Salinity effect on residual dry matter
Salinity Level	Mean	Mean
4	0/39 a	0/48 a
20	0/21 b	0/23 b
30	0/11 c	0/13 c
40	0/04 c	0/02 c

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level

Table 5: Means comparison of different levels of aridity treatments on dry matter production and residual dry matter

	Salinity effect on dry matter production	Salinity effect on residual dry matter
Aridity Level	Mean	Mean
Fc	0/27 a	0/39 a
-8 bars	0/15 b	0/22 b
-12 bars	0/09 c	0/10 c

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level

Table 6: Results of the analysis of variance (ANOVA) of effects of different grazing treatments on dry matter production and residual dry matter

	Salinity effect on dry matter production	Salinity effect on residual dry matter
Grazing levels	Mean	Mean
0% cutting	0/20 a	
	0/41 a	
25% cutting	0/27 b	0/29 b
50% cutting	0/15 c	0/22 c
75% cutting	0/05 d	0/07 d

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level

Table 7: Results of the analysis of variance (ANOVA) of interaction effects of salinity×aridity treatments on dry matter production and residual dry matter

		Salinity×aridity intraction on dry matter production	Salinity×aridity intraction on dry matter production
Aridity levels	Salinity levels (dS/m)	Mean	Mean
Fc	4	0/44 a	0/58 a
-8 bars	4	0/32 b	0/54 a
-12 bars	4	0/16 c	0/38 b
Fc	20	0/35 b	0/57 a
-8 bars	20	0/18 c	0/40 b
-12 bars	20	0/11 c	0/33 c
Fc	30	0/07 d	0/14 d
-8 bars	30	0/01 d	0/08 de
-12 bars	30	0 d	0 e
Fc	40	0 d	0 e
-8 bars	40	0 d	0 e
-12 bars	40	0 d	0 e

Means of five replicated pots are given and different letters in the same column are significantly different at the 0.05 probability level

Table 8: Results of the analysis of variance (ANOVA) of main effects and interactions effects of different species, salinity, aridity and grazing treatments on survivals scores of *Agropyron elongatum*, *Kochia prostratea* and *Puccinellia distans* at various stages of phenology

		Main effects and interactions effects on survivals scores of *Agropyron elongatum*, *Kochia prostratea* and *Puccinellia distans*		
Sources of Variations	df	MS	F	P
Species	2	0/67	101/17	*/00
Salinity Level	3	0/53	85/14	*/00
Aridity level	2	0/42	98/12	*/00
Grazing level	3	0/48	99/14	*/00
Species×Salinity	6	0/19	63/19	ns 0/06
Species×Aridity	4	0/37	53/11	ns 0/07
Species×Grazing	6	0/41	68/12	ns 0/05
Salinity×Aridity	6	0/11	18/32	*/00
Salinity×Grazing	9	0/29	42/19	ns 0/05
Aridity×Grazing	6	0/34	53/10	ns 0/06
Species×Aridity×Grazing×Salinity	96	0/06	7/18	ns 0/07

* significant at 0/05 MS: Mean of Squares F: Fisher Statistics
ns: not significant at 0/05 df: degree of freedome P: significant Level

Table 9: Means comparisons of the species types, Salinity levels, Aridity levels and simulated grazing levels effects on survival scores

	Species types	Mean
Species types effects on survival scores	*Agropyron elongatum*	0/35 a
	Puccinellia distans	0/29 a
	Kochia prostrata	0/18 b
	Salinity levels	Mean
Salinity levels effects on survival scores	Control	0/87 a
	20	0/54 b
	30	0/26 c
	40	0/06 d
	Aridity levels	Mean
Aridity levels effects on survival scores	Fc	0/68 a
	-8 bars	0/43 b
	-12 bars	0/12 c
	Simulated grazing levels	Mean
Simulated grazing levels effects on survival scores	0% cutting	0/64 a
	25% cutting	0/61 a
	50% cutting	0/59 a
	75% cutting	0/58 ab

Table 10: Results of the analysis of variance (ANOVA) of interaction effects of salinity×aridity treatments on survival scores

		Salinity×aridity interaction on survival scores
Aridity levels	Salinity levels (dS/m)	Mean
Fc	4	0/82 a
-8 bars	4	0/61 b
-12 bars	4	0/50 c
Fc	20	0/39 d
-8 bars	20	0/27 e
-12 bars	20	0/11 g
Fc	30	0/18 f
-8 bars	30	0 h
-12 bars	30	0 h
Fc	40	0 h
-8 bars	40	0 h
-12 bars	40	0 h

Results of the analysis of variance (ANOVA) main effects and interactions effects of different species, salinity, aridity and grazing treatments on survivals scores of *Agropyron elongatum*, *Kochia prostratea* and *Puccinellia distans* at various stages of phenology are shown in Table 8 ($p < 0.05$).

Means comparisons of the species types, Salinity levels, Aridity levels and simulated grazing levels effects on survival scores is showed in Table 9.

Results of the analysis of variance (ANOVA) of interaction effects of salinity×aridity treatments on survival scores are shown in table 10 ($p < 0.05$).

DISCUSSION

The highest mean of dry matter (gr) and the residual dry matter (gr) were seen in control treatments of salinity, aridity and grazing. This was seen in all three concerned species (*Agropyron elongatum*, *Kochia prostrata* and *Puccinellia distans*) (Tables 3, 4, 5 and 6). The minimum amount of dry matter (gr) and the residual dry matter (gr) mean were seen in treatments with 40 dS/m salinity level, 75% cutting level and -12 bars for aridity (Tables 4 and 5). The seeds survival scores is zero in treatments with 40 dS/m salinity and -12 bars aridity (Table 10). These results are in agreement with 22, 23 24 25 and 26 research results. The 22, 23 24 25 and 26 studies report that the interaction of salinity and aridity is due to water availability limitation uptake. Water uptake limitation is due to plant death. Tolerance threshold for germination and establishment of *Agropyron elongatum* under aridity× salinity interaction effect is -12 bar aridity and 40 dS/m salinity treatment. Threshold for germination and establishment of Kochia prostrata and Puccinellia distans under aridity× salinity intraction effect is -8 bar aridity and 30 dS/m salinity treatment (Figures 1, 2 and 3).

25% cutting caused increment in residual dry matter. Increasing grazing level caused increment in dry matter production compare to 0% cutting (Figures 1, 2 and 3). These results are in agreement with [32] reports that said low grazing or conservative grazing due to increment in residual dry matter.

The average soil surface salinity of Incheh Boroun is 28 dS/m [9]. Therefore, *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* can survive in a saline soil similar to that of the area in Incheh Boroun saline rangeland. However, for introducing any plant species to this area and its adaptation in this region, the ecological, edaphic and climatic conditions of Incheh Boroun must be taken into consideration.

Kochia prostrata could not survive at 40 dS/m salinity level (Figure 1). Although *Kochia prostrata* is a halophytic plant species [21], high salinity levels of the study induced deleterious effects on the growth of this species. There are a few studies found on the tolerance of rangeland plants to salinity [16, 17, 24]. Rangeland halophytic plant species can tolerate different degrees of salinity stresses. Therefore, the rangeland halophytic plant species must be classified into various salinity tolerance levels.

An important finding was that *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* could survive at 30 dS/m and *Agropyron elongatum* and *Puccinellia distans* could survive at 40 dS/m. Other researchers have not observed these plants growing at these ranges of salinity [11-21].

In our study, *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* exhibited different degrees of salt tolerance. Other researchers have not observed these plants growing at these ranges of salinity stresses [11, 21].

Under salinity stress conditions, nutrient and water absorption by roots and shoot growth are reduced [25]. Thus, there are significant differences between the growth of different species in the same salinity level.

The highest survival scores have been seen in *Agropyron elongatum*. However, the lowest survival scores have been seen in *Kochia prostrata* (Table 5). This is supported by other investigators [11-21, 30, 31].

The ecological nature of *Puccinellia distans*. *Puccinellia distans* is a hydrohalophytic plant species [26].

This threshold salinity level can be used only to determine soil salinity in agricultural lands. Therefore, it is necessary to establish a new local soil salinity classification and threshold on the saline rangelands.

The results of our study showed that the growth of *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* have been curtailed in this saline soil rangeland. Thus, these species are Facultative halophytic plant species [12.

CONCLUSIONS

Overall, considering the results of this study, it could be concluded that among the studied plant species, *Agropyron elongatum* exhibited the maximum salt, aridity and grazing tolerance and *Kochia prostrata* showed the minimum salt tolerance.

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