EFFECT OF SALANITY ON GERMINATION OF *TRIFOLIUM REPENS, DACTYLIS GLOMERATA* AND *MEDICAGO SATIVA*.

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Abstract

Effect of salinity on three imported seeds i.e. *Trifolium repens, Dactylis glomerata* and *Medicago sativa* was studied during the present study. Experiments were conducted in salinity induced soils. Seed germination and speed of germination were recorded. Study showed in higher salinity exhibit no germination. In garden soil *Trifolium repens* showed highest percentage of germination i.e. 56.66% ±1.55 while percentage of germination of *Medicago sativa* and *Dactylis glomerata* was 50% ±1.00 and 37% ± 1.155 respectively. It was also observed that *Dactylis glomerata* is highly salinity sensitive in comparison to other two species.

Introduction

Successful establishment of any plant depends upon its germination which is a critical period of plants life. Seed germination depends upon various factors such as light, temperature, water availability, salinity, etc (Khan *et al.,* 1984; Khan 1987; Al-Ahmed and Kafi, 2007; Parez-Garcia *et al*., 2007; Guma *et al*., 2009; Ruano *et al*., 2009).

Increasing soil salinity is a major plant stress in Agro-ecosystem worldwide. Salinity in agricultural ecosystem directly affects plant production. According to Munns, (1993), when plants are exposed to salinity, they immediately experience osmotic stress due to low water potential of the substrate. Soil salinity is considered as one of the major environmental stress which affects plant growth and productivity leading to considerable losses in crop productivity Azeem and Ahmed (2011). Chemical composition of soil/substrate and availability of nutrients are very important factors of plant growth and development, especially in conditions of increased salinity. Sufficient supply of nutrients promotes not only plant growth, but also plant tolerance to different diseases and stress conditions Bergmann and Naubert (1976); Marschner, (1995).

Salinity has affected more than 800 million hectare of land throughout the world which is almost 6% of the worlds total land area. Salinity is also a major problem by which 6.8 mha (33%) area of cultivated land of Pakistan is affected (Anon., 2008). Out of 20.36 million hectare of cultivable land 6.3 million is affected by salinity (Qureshi and Barret-Lennard, 1998).

However, no one in Pakistan worked on salinity effect on these pasture species (*Medicago sativa, Trifolium repens* and *Dactylis glomerata).*The purpose of this study was to explore salinity tolerance of *Medicago sativa, Trifolium repens* and *Dactylis glomerata*. The seeds of these pasture species were imported from New Zealand.

Materials and Methods

The seeds were kept in dried glass bottles at constant temperature i.e. 25 C. Seeds of similar sizes were selected for each experiment and germinated under different salinity levels. Three replicates were set for each treatment and 10 seed were placed in each Petri dish. Highly Saline Soil was collected from Federal Urdu University for Arts Science and Technology Gulshan-e-Iqbal Campus and Garden loam soil was mixed with saline soil with different ratios. Each petri dish had 20 g the soil. The salinity levels were achieved by adding garden soil with a highly saline soil in different proportions (91:9, 92:8, 93:7, 94:6, 95:5, 96:4, 97:3, 98:2 and 99:1) pH, conductivity, TDS, and salinity level of these soils were recorded using multimeter. Germination of seeds was recorded after every 24 hours till maximum germination was obtained. Seeds were considered to have germinated at the emergence of radicle (Bewley and Black, 1994).

Statistical Analysis: A speed of germination index (S) for each species was determined by following formula (Khandakar and Bradbeer 1983).

$$\mathbf{S= [\ N_1/1 + N_2\ /2 + N_3/3 +\ldots\ldots\ldots + N_n/N\ ]\ X\ 100}$$

where N1, N2, N3…..N= total number of seeds in a treatment which germinated on day 1, 2, 3…N.The index S ranges from 0 (if no seed ever germinated) to 100 (if all seeds germinated on the first day). The program GERMSPD was developed by Syed Shahid Shaukat was used. Analysis of variance was conducted to compare means.

Results and Discussion

Table 1. pH, Temperature, Conductivity, TDS and Salinity level of Garden loam, Saline Soil and their mixtures in different ratios.

Salinity ratio	pH	Temperature (C)	Conductivity	TDS (ppm)	Salinity (PPT)
Garden soil (control)	7.98	26.45	380 µ/cm	189	0.18
99:1 (T1)	7.77	26.79	489 µ/cm	263	0.25
98:2 (T2)	7.79	26.84	527 µ/cm	424	0.41
97:3 (T3)	7.81	26.65	974 µ/cm	533	0.53
96:4 (T4)	7.89	26.89	1067 µ/cm	619	0.61
95:5 (T5)	7.9	26.45	1340 µ/cm	670	0.67
94:6 (T6)	7.92	26.71	1572 µ/cm	786	0.79
93:7 (T7)	7.93	26.71	1593 µ/cm	796	0.8
92:8 (T8)	7.95	26.61	1967 µ/cm	982	0.99
91:9 (T9)	7.95	26.63	2461 µ/cm	1233	1.26
Saline soil	7.32	26.82	13.68 ms/cm	6889	7.94

Table 1. shows the salinity levels of pure Garden loam (Control) and Highly Saline soil (T10) and their different proportions garden loam: highly saline Soil (99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9). The Table shows that saline soil has highest salinity level which is 7.94 PPT . While pure Garden loam has very low salinity level which is 0.18 PPT. The salinity levels of mixed soils varied from 489-246 µS/cm.

Table 2. Germination percentage and speed of germination of *Trifolium repens*, *Medicago sativa* and *Dactylis glomerata* at various salinity levels.

Treatment	*Trifolium repens*		*Medicago sativa*		*Dactylis glomerata*	
	Germination percentage	Speed of germination	Germination percentage	Speed of germination	Germination percentage	Speed of germination
Garden soil (Control)	56.66 ± 1.54701	19.66	50 ±1.00	13.23	37 ± 1.154701	4.68
99:1 (T1)	26.66 ± 0.57735	7.055	25.2 ± 0.7735	6.722	23.33 ± 0.57735	4.01
98:2 (T2)	16.66 ± 0.57735	4	13.33 ± 1.5470	3.277	16.66 ± 0.57735	2.77
97:3 (T3)	10 ± 1.00	2.05	13.33% ±0.57735	3.611	6.66 ±0.57735	0.95
96:4 (T4)	6.66 ± 0.57735	1.94	10 ±0.00	2.77	0 ±0.00	0
Saline soil (T10)	0 ±0.00	0	0 ±0.00	0	0 ±0.00	0

Table 2. Show germination percentage and speed of germination of three plant species under saline soil, garden soil and their various combinations. All three species showed significant effect of salinity ($F = 18.25$, $p<0.001$; $F = 13.72$, $p<0.001$; $F = 8.14$, $p<0.01$ for *Trifolium repens*, *Medicago sativa* and *Dactylis glomerata*, respectively). In salinity treatments from T1 to T5 no germination was recorded. Highest percentage of germination of *Trifolium repens* was obtained in Garden loam which is 56.66% ± 1.54 when Speed of germination was 19.66.Germination as well as germination velocity regularly declined from treatment (T1) to (T4) in case of *Trifolium repens* and *Medicago sativa Dactylis glomerata* could not germinate in (T4), probably due to its greater sensitivity to salinity.

Seeds of *Trifolium repens* in garden soil (Fig. 1) started to germinate on 2nd day and highest percentage of germination was obtained on 4th day which was 56.66% ± 1.55 after that no seed germinated. Fig.2. shows the per day germination percentage of *Trifolium repens* in 96% garden loam: 4% saline soil. Germination started on 3rd day and highest percentage of germination was obtained on 4th day which was 6.66% ± 0.58 after which germination ceased. Fig.3. shows the germination percentage per day of *Trifolium repens* in 97% garden loam: 3% saline soil. Germination started on 4th day and maximum germination was obtained on 6th day which was 10% ± 1.00. After that day no germination was recorded. Fig.4. shows the germination percentage per day of *Trifolium repens* in 98% garden loam: 2% saline soil. Germination started on 3rd day and maximum germination

was obtained on 6th day which was 16.66% ± 0.58. After that germination ceased. Fig.5. shows the germination percentage per day of *Trifolium repens* in 99% garden loam: 1% saline soil. Germination started on 3rd day as this treatment has lowest salinity level so it showed highest germination percentage among all concentrations. Maximum germination was observed on 6th day which was 16.66% ± 0.58. After that germination ceased.

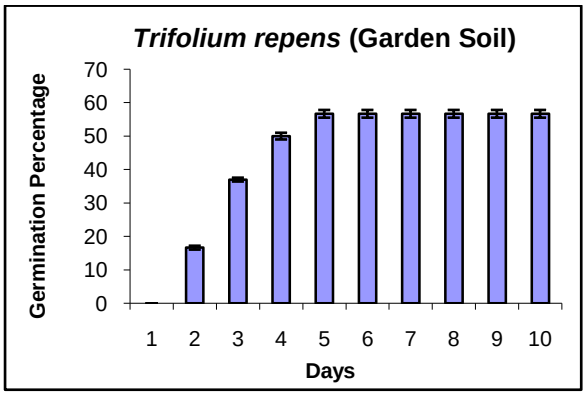

Fig.1. Germination percentage as function incubation period of *Trifolium repens* in (Garden soil 96%: Saline soil 4%)

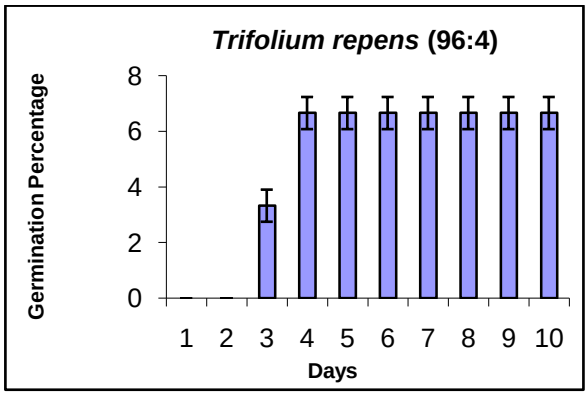

Fig.2. Germination percentage as function incubation period of *Trifolium repens* in Garden soil

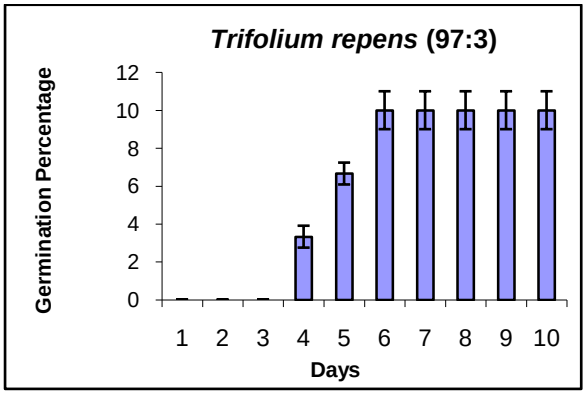

Fig.3. Germination percentage as function incubation period of *Trifolium repens* in 97%: Saline 3%).

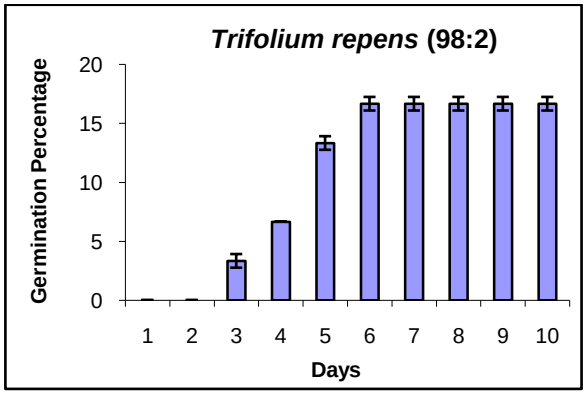

Fig.4. Germination percentage as function incubation period of *Trifolium repens* in (Garden soil (Garden 98%: Saline 2%).

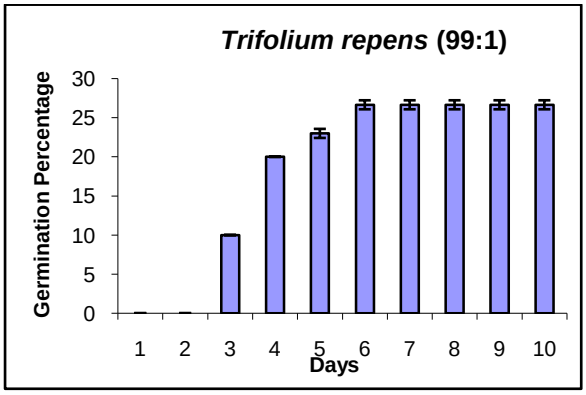

Fig.5. Germination percentage as function of incubation period of *Trifolium repens* in (Garden 99%:Saline 1%).

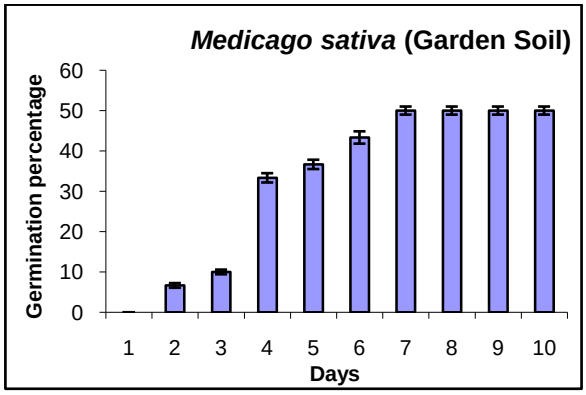

Fig.6. Germination percentage as function of incubation period of *Medicago sativa* in (Garden soil 96%:Saline soil 4%).

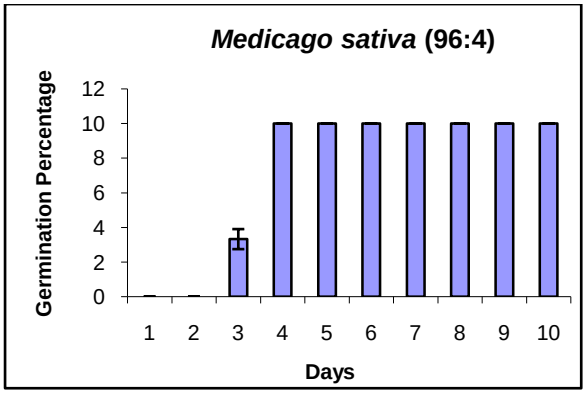

Fig.7. Germination percentage as function incubation period of *Medicago sativa* in Garden soil.

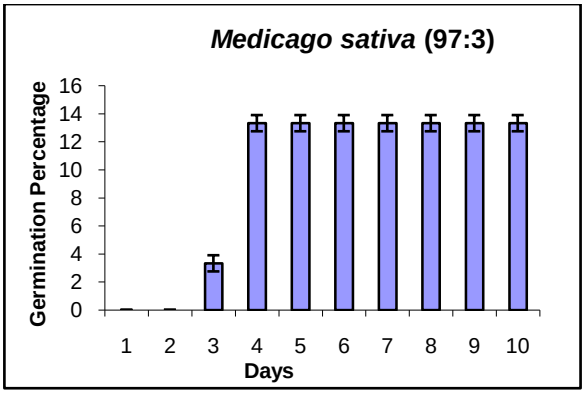

Fig.8. Germination percentage as function of incubation period of *Medicago sativa* in 97%:Saline soil 3%).

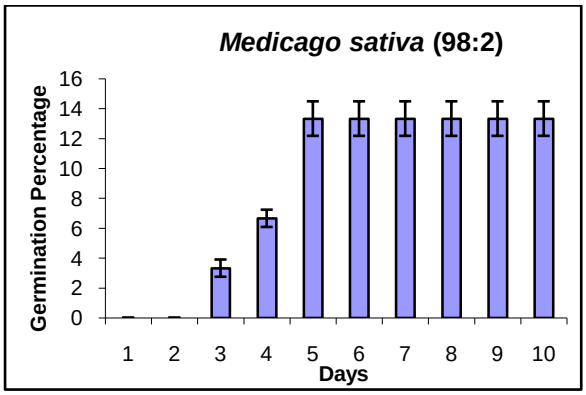

Fig.9. Germination percentage as function of incubation period of *Medicago sativa* in Garden soil (Garden soil 98%: Saline soil 4%).

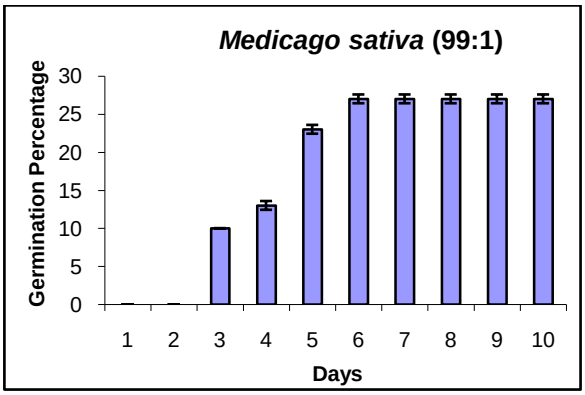

Fig.10. Germination percentage as function of incubation period of *Medicago sativa* in (Garden soil 99%: Saline soil 1%).

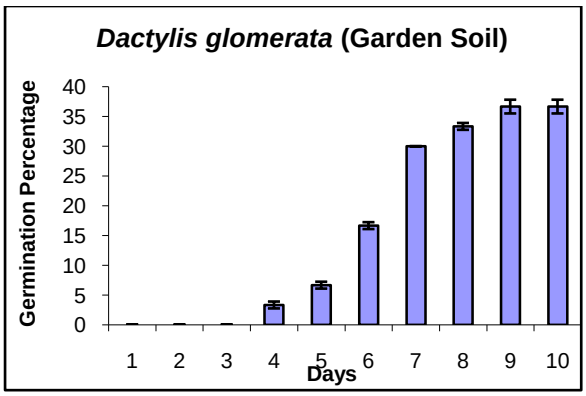

Fig.11. Germination percentage as function of incubation period of *Dactylis glomerata* in (Garden soil 97%: Saline soil 3%).

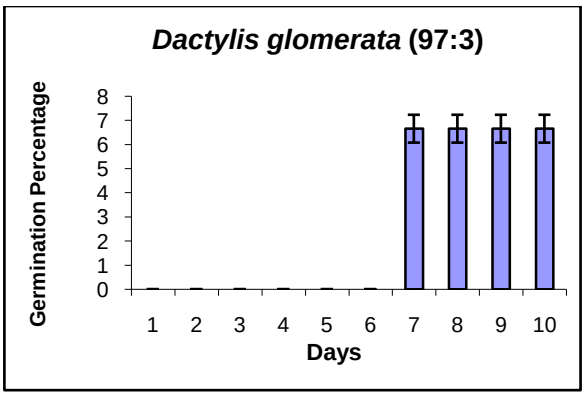

Fig.12. Germination percentage as function of incubation period of *Dactylis glomerata* in Garden soil.

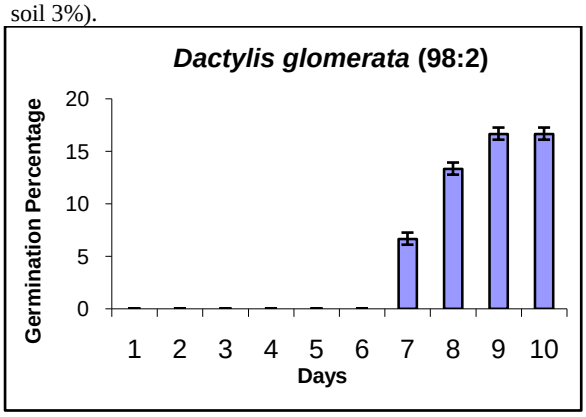

Fig.13. Germination percentage as function of incubation period of *Dactylis glomerata* in Garden soil98%: Saline soil 2%).

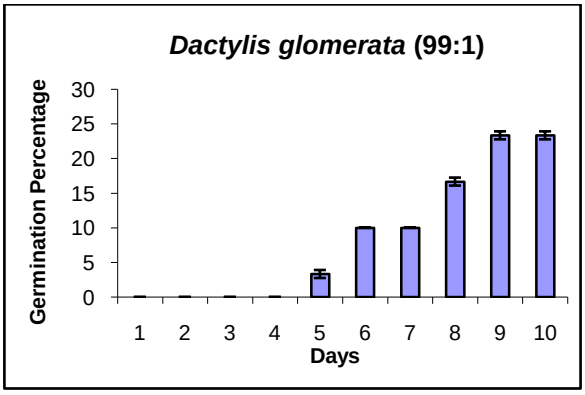

Fig.14. Germination percentage as function of incubation period of *Dactylis glomerata* in(Garden soil 99%: Saline soil 1%).

Table 2 also shows germination percentage and speed of germination of *Medicago sativa* under saline soil, garden soil and their different concentrations i.e. garden loam: saline (96:4, 97:3, 98:2, and 99:1). Like previous plant in saline soil up to 95:5 concentrations no germination was recorded. Highest percentage of germination of *Medicago sativa* was obtained in Garden loam which is 50% ± 1.00. Speed of germination was 13.23. Percentage of germination in ratio 96:4 was 10% ± 0.00 with 2.77 speed of germination. Concentration 97:3 shows 10% germination ±0.58. While speed of germination for this ratio was 3.611. 98:2 ratio showed 13.33% germination ± 1.55 and speed of germination was 3.277 In concentration 99:1 *Medicago sativa* showed highest percentage of germination from 96:4 to 99:1 which is 25.2% ±0.58 with 6.722 speed of germination but this germination percentage is almost half as compared to garden soil germination.

Fig.6. Show the percentage of germination per day of *Medicago sativa* in garden loam. Seeds started to germinate on 2nd day and highest percentage of germination was obtained on 7th day which was 50% ± 1.00 after that no seed germination was observed. Fig.7. show the per day germination percentage of *Medicago sativa* in 96%garden loam: 4% saline soil. Germination started on 3rd day and highest percentage of germination was obtained on 4th day which was 10% ± 0.00 after which germination ceased. Fig.8. show the germination percentage per day of *Medicago sativa* in 97% garden loam: 3% saline soil. Germination started on 3rd day and maximum germination was obtained on 4th day which was 13.33% ± 0.58. No seed germinated after that day was seen. Fig.9. show the germination percentage per day of *Medicago sativa* in 98% garden loam: 2% saline soil .Germination started on 3rd day and maximum germination was obtained on 5th day which was 13.33% ± 1.155. After that germination ceased. Fig.10. show the germination percentage per day of *Trifolium repens* in 99% garden loam: 1% saline soil. Germination started on 3rd day as this treatment has lowest salinity level so it showed highest germination percentage from 96:4 to 99:1. Maximum germination was obtained on 6th day which was 27% ± 0.58. After that germination ceased.

Germination percentage and speed of germination in *Dactylis glomerata* under saline soil, garden soil and their different concentrations garden loam: saline 97:3, 98:2, and 99:1 is given in Table 2. In saline soil and concentrations of 991:1 to 96:4 salinity germination was significantly reduced ($p<0.001$). Highest percentage of germination of *Dactylis glomerata* was obtained in garden loam which is 37% ± 1.54701. Speed of germination was 4.68. Lowest percentage of germination was recorded in the ratio of 97:3 garden loam: saline soil which was 6.66% ± 0.57 with 0.95 speed of germination. Concentration of 98:2 also shows 16.66 ± 0.57 % germination while speed of germination for this ratio was 2.77. At a concentration of 99:1garden loam : saline soil, *Dactylis glomerata* showed highest percentage of germination which is 23.33% ±0.57735 with 4.01 speed of germination.

Fig.11. Show the percentage of germination per day of *Dactylis glomerata* in garden soil. Seeds started to germinate on 4th day and highest percentage of germination was obtained on 9th day which was 36.66% ± 1.55 after that no seed was germinated. Fig.12. show the per day germination percentage of *Dactylis glomerata* in 97%garden soil: 3%saline soil. Germination started and also stopped on 7th day and maximum germination percentage was 6.66% ± 0.57735. No seed germinated after that day. Fig.13. show the germination percentage per day of *Dactylis glomerata* in 98% garden soil: 2% saline soil. Germination started on 7th day and maximum germination was obtained on 9th day which was 16.66% ± 0.58. After that germination ceased. Fig.14. show the germination percentage per day of *Dactylis glomerata* in 99% garden soil: 1% saline soil. Germination started on 5th day as this treatment has lowest salinity level so it showed highest germination percentage. Maximum germination was obtained on 9th day which was 23.33% ± 0.58. After that germination ceased.

Table 3(a). Analysis of Variance; ***Trifolium repens.***

Source	DF	SS	MS	F-Value	P-Value
Treatment	4	48.667	12.1667	18.25	0.001
Error	10	6.667	0.667		
Total	14	55.333			

Table 3(b). Analysis of Variance; ***Medicago sativa.***

Source	DF	SS	MS	F-Value	P-Value
Treatment	4	32.933	8.2333	13.72	0.001
Error	10	6.000	0.6000		
Total	14	38.933			

Table 3(c). Analysis of Variance; ***Dactylis glomerata.***

Source	DF	SS	MS	F-Value	P-Value
Treatment	3	14.250	4.7500	8.14	0.001
Error	8	4.667	0.5833		
Total	11	18.917			

Table 3 a,b,c show the results of analysis of variance and their significance level.
The effect of salinity level was found to be significant. ($F=18.25$, $p<0.001$) in *Trifolium repens*, ($F=13.72$, $p<0.001$) in *Medicago sativa*, and ($F=8.14$, $p<0.001$) in *Dactylis glomerata.*

According to Debez *et al*. (2004) germination is one of the most critical periods in the life cycle of plant; however due to salt stress low water potential develop which inhibits seed germination. In plants, level of salinity tolerance is different during different stages of development from germination to mature plant (Ungar,1995; Khan and Ahmad, 1998). However, salinity tolerance is a very important phenomenon during germination. It is also very important for plant establishment in varying environmental and geological regions around the world (Ungar, 1995).

Medicago sativa was markedly influenced by mixed salt-alkali stress, the interaction of salinity significantly affected germination rate of alfalfa seed (Zhanwu *et al*., 2011).

Medicago sativa is best adapted to soils having pH 6 and 8. It highly tolerant to drought and fire but does not tolerate poor soil drainage, flooding , salinity and shading (Hulten, 1968).

Dactylis glomerata is severely affected by salinity and lower salinity levels of irrigation water have a positive and significant impact on most of the characteristics (Aligolizadeh and Eimani, 2011).

There are different opinions about the tolerance of *Trifolium repens* to increased salinity. *Trifolium repens* is often considered to be a salt-sensitive pasture legume (Ghonzalez-Murua *et al*., 1985; Ghassemi-Golezani *et al*., 2009) and to be absent from salt rich soils (Burdon, 1983). However, salt tolerant cultivars, genotype and populations of *Trifolium repens* have been identified (Ab-shukor *et al*., 1988; Roger and West, 1993; Rogers *et al.,* 1997; Wang *et al*., 2010). White clover (*Trifolium repens*) is a commonly cultivated legume with good ground cover potential. Soil salinization is also important issue associated with low water resources and highly urbanized areas in arid and semi arid climatic conditions .(Asci, 2011; Vahdati *et al*., 2012; Saberi *et al*., 2013).

According to Duan (2004) *Chenopodium glaucum* show better germination under non saline and control but increase in salinity inhibited seed germination. Miller and Champan (1978) worked on germination responses of *Agropyron elongatum, Festuca arundinacea* and *Phalaris canariensis* to different concentrations of six salts including NaCl, Na_2SO_4, Mg_2Cl_2, Mg_2SO_4, KCl, K_2SO_4 and found similar results in form of decreasing rate of germination in increasing salt concentrations.

On the basis of above results it is evident that the three pasture species are highly sensitive to salinity in which *Dactylis glomerata* is the most salt- sensitive compared to the other two species. *Trifolium repens* shows less sensitivity compared to *Medicago sativa* and *Dactylis glomerata* though all three species are sensitive to salinity and they cannot be recommended for cultivation in the pasture land of Sindh which are saline to varied extent. However they may be suitable to some non saline pastures of Punjab and KPK.

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