ELECTROLYTE LEAKAGE AND RELATIVE WATER CONTENT AS AFFECTED BY ORGANIC MULCH IN OKRA PLANT (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH) GROWN UNDER SALINITY

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Abstract

The present study was carried out to determine the effect of organic mulch with or without gypsum amendment on relative water content (RWC) and electrolyte leakage (EL) in leaves of okra (lady finger) plant grown under saline water irrigation. The plants were grown in pots irrigated with S1 (EC_{iw} = 2.0 dSm^{-1}), S2 (EC_{iw} = 4.2 dSm^{-1}) and non-saline control (EC_{iw} = 0.5 dSm^{-1}). Increased EL was noted under salinity without mulch treatments, whereas, application of mulch reduce EL under all salinity treatments. Greater reduction was recorded under mulch amended with gypsum as compare to mulch alone. RWC in leaves of okra was proportionally decreased with increasing salinity. Organic mulch alone or amended with gypsum showed increased RWC under all salinities as compare to without mulch treatments.

Results of the present study suggested that application of organic mulch to the soil surface can improves salinity tolerance up to certain extent and the mulch with gypsum up to greater extent by improving RWC and reducing EL in plant which are responsible for improvement of growth as well.

Introduction

Salinity is one of the major factor which causes inhibitory effects on plant growth. The reduction in growth under saline conditions is more severe in arid and semi-arid regions (Rengasamy, 2006; Bonilla *et al*., 2004) due to adverse effects on metabolic and physiological processes (Krishnamurthy *et al*., 2007; Tester and Devenport, 2003).

The key function of plasma membrane is to act as barrier for the transporting molecules because it is controlled through protein channel along with carrier and phospholipids present in the membrane (Mansour, 1997, Wang *et al.,* 2008). Many scientists agree that the plasma membranes are the primary site of ion specific salt injury (Mansour and Salama, 2004). Therefore, electrolyte leakage (EL) from the plasma membrane has been reported one of the most important selection criterion for identification of salt tolerant plants (Ashraf & Ali, 2008 and Tiwari *et al*., 2010). Relative water content (RWC) is another aspect to examine the water balance in the plants (Gonzalez and Gonzalez-Vilar, 2001). Many scientists proposed that water balance regulation in plants favors the avoidance mechanism and diluting the effects of ionic toxicity under salt stress (Ashraf, 2004 and Noreen *et al*., 2010).

Spreading of organic matter on the soil surface is a known practice. It is a good technique to reduce capillary rise of salts and maintain soil moisture in arid regions. Saeed and Ahmad (2013) found that mulch composed of sawdust, grass clipping and cow dung manure retained moisture content for about 21 days up to 50 % of its full saturation. Increase soil water content has also been reported under mulch treatment by Tejedor *et al*., (2003; 2007). Use of mulch helped to improve plant growth by lowering down the salinity of rhizosphere (Pang *et al*., 2010; Saeed and Ahmad, 2009). Calcium being one of the main constituent of gypsum provides essential macro element directly involve in functioning of membrane. The role of calcium in membrane stability and lowering EL under salt stress to improve tolerance index of plants is also reported in literature (Hirshi, 2004). Addition of calcium chloride in hydroponics is found to reduce relative EL in the rice seedlings growing under 200 mM NaCl (Cha-um *et al*., 2012).

Okra, (*Abelmoschus esculentus* (L.) Moench) ladys' finger or bhindi is a popular vegetable with medicinal importance belongs to family Malvaceae. It is rich in minerals, carbohydrate, protein, fat and phenols (Jideani and Adetula, 1993). Its mucilage is used as thickener in food industries. Polysaccharides extracted from young okra fruits blocked the adhesion of *Helicobacter pylori* which cause stomach cancer (Wittschier *et al*., 2007). Additionally, the dry plants stems are the good source of fibers for paper industry (Baloch, 1994). Variation in salinity tolerance of okra genotype is found from sensitive (ECe 1.0 dSm^{-1}, Bresler *et al*., 1982) to the tolerant varieties (ECe 3.48 dSm^{-1}, Unlukara, *et al*., 2008).

The aim of present study is to evaluate the effectiveness of organic mulch alone and with gypsum as supplement for preventing loss of membrane permeability and maintaining relative water content in okra plants grown under saline conditions.

Materials and Methods

Experimental design: The earthen pot experiment was conducted at Biosaline Research Nursery, Department of Botany at University of Karachi. Surface sterilized seeds of okra were sown in earthen pots having basal hole containing 20 Kg sandy loam and cow dung manure (9:1). The pots were initially irrigated with tap water (non-saline control, EC_{iw} = 0.5 dSm^{-1}) till the seedlings reached up to two leaf stage. The salinity treatments were started later through irrigation of the water of two salinity levels made by dissolving sea salts till they reached to the EC, S1 = 2.0 dSm^{-1} and S2 = 4.2 dSm^{-1}. Four inch thick layer of the mulch was spread on the soil surface as mulch treatment. The mulch was prepared by mixing of grass (*Cyanodon dactylon*) clipping, sawdust (*Cedrus deodara*) and cow dung manure in ratio of 1:2:1, soaked in tap water and kept for about 6 months for partial decomposition. There were three mulch treatment (non-mulch control, mulch alone and mulch+ 0.2% gypsum) and three replicates for each treatment.

The EC of rooting medium were determine in saturated soil and mulch extracts separately by 4510 conductivity meter (JENWAY), (Richards, 1954).

Plant material and analysis: EL and RWC was recorded from the third leaf down the shoot apex after sixty days to salinity and mulch treatments.

Electrolyte Leakage (EL) was determined by the method of Lutts, *et al*., (1996) with little modification. One cm^2 pieces of leaf without midrib or major veins were placed in test tubes containing 10 mL of distilled water and record the EC at room temperature. The same was kept at 40°C for half an hour and record EC after cool and then boiled the same at 100°C for another half an hour. EL was determined by the formula,

Electrolyte Leakage (%) = (EC at 40°C – EC at room temp) / (EC at 100°C) × 100.

Relative water content: (RWC) in leaf (one cm^2 piece without midrib) was estimated by the method of Barrs and Weatherley (1962) by using the formula,

RWC (%) = (Fresh Weight – Oven dry weight) / (Turgid weight – Oven dry weight) × 100.

Statistical analysis of variance (TWO-WAY ANOVA) and Duncan's multiple range tests (DMRT) was performed by costat at $LSD_{0.05}$.

Result and Discussion:

Effect of Mulch and Salinity on EC of the soil: Electrical conductivities of the saturated soil and mulch extracts at grand period of plant growth is presented in Table 1, which shows that increase in EC is directly proportional with increasing salts in irrigation water. In case of soil dressed with surface mulch, EC of the soil beneath the mulch is lower than the EC of mulch spread on it. Similar trend is observed in EC of the soil under mulch with gypsum. Mulch with gypsum results comparatively greater EC of the soil and mulch (mulch with gypsum) as well which is presumably due to the addition of gypsum. Little increase in EC of soil and mulch was recorded due to addition of gypsum as EC of the aqueous solution of pure gypsum at 25°C is 2.2 dSm^{-1} (Richards, 1954).

Table 1. Electrical conductivities (EC) of the saturated soil and mulch extracts after sixty days to the treatments.

Salinity	Mulch Treatment	EC of Soil dSm^{-1}	EC of Mulch dSm^{-1}
Control (Non saline) EC iw = 0.5 dSm^{-1} (irrigated with non-saline solution)	Soil alone	1.8±0.058	-
	Mulch alone	1.5±0.008	1.8±0.058
	Mulch+Gypsum	2.1±0.173	2.9±0.153
S_1 EC iw = 2.0 dSm^{-1} (irrigated with 0.15% sea salt solution)	Soil alone	3.1±0.230	-
	Mulch alone	2.1±0.230	3.2±0.100
	Mulch+Gypsum	3.6±0.173	4.2±0.058
S_2 EC iw = 4.2 dSm^{-1} (irrigated with 0.3% sea salt solution)	Soil alone	4.3±0.115	-
	Mulch alone	3.2±0.115	4.6±0.058
	Mulch+Gypsum	3.4±0.180	5.8±0.313
	F values	40.875 P < 0.001	255.986 P < 0.001

Mulch accumulates salts and act as sieve or barrier for the irrigating saline water and prevent leaching of excess salts to the rhizosphere. Desalinization of soil by application of surface organic mulches was also noted by Dong, *et al*., (1996). Our results are also supported by the observation Abro *et al*., (2007) and Saeed and Ahmad (2009). Addition of organic matter and gypsum to the surface soil has decreased dispersion and EC down to the subsoil, compared to the addition of gypsum alone (Vance *et al*., 1998).

Effect of Salinity on Leaf EL: Significant increase in electrolyte leakage ($F = 42.157$, $P < 0.0001$) from okra leaf grown under increasing salinity of irrigation water (EC_{iw} 2.0 dSm^{-1} and 4.2 dSm^{-1}). Fig. 1 showed highest amount of EL was observed in plants irrigated with saline water (EC_{iw} 4.2 dSm^{-1}) as compared to lower salinity (EC_{iw} 2.0 dSm^{-1}) and non-saline control (EC_{iw} 0.5 dSm^{-1}). Increase in EL has been documented by Latrach *et al*., (2014) in alfalfa, Baninasab and Baghbanha, (2013) in cucumber, Salwa, (2010) in peanut and Khurram *et al*., (2009) in pepper. EL, however, appears to be related with the salt tolerance of a species. Ali et al., (2013) found no significant increase in EL in *Sorgham bicolor* cultivars up to 100 mM NaCl ($ECiw = 14.2$ dSm^{-1}). Some workers have reported increase in electrolyte leakage in plants exposed to salinity (Dkhil and Denden, 2012; Kaya et al., 2001a and 2001b).

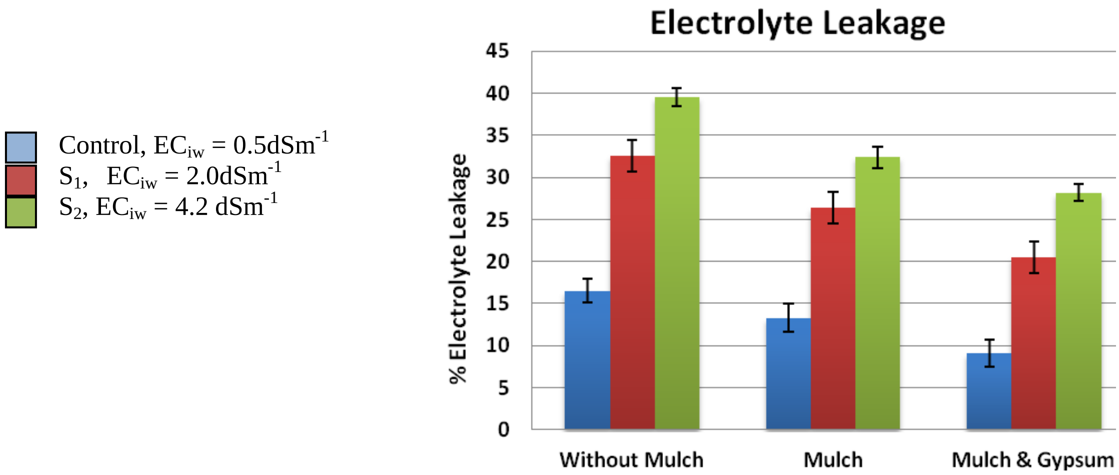

Fig. 1. EL in leaf of Okra grown under sea salt concentrations at soil supplemented with mulch with or without gypsum.

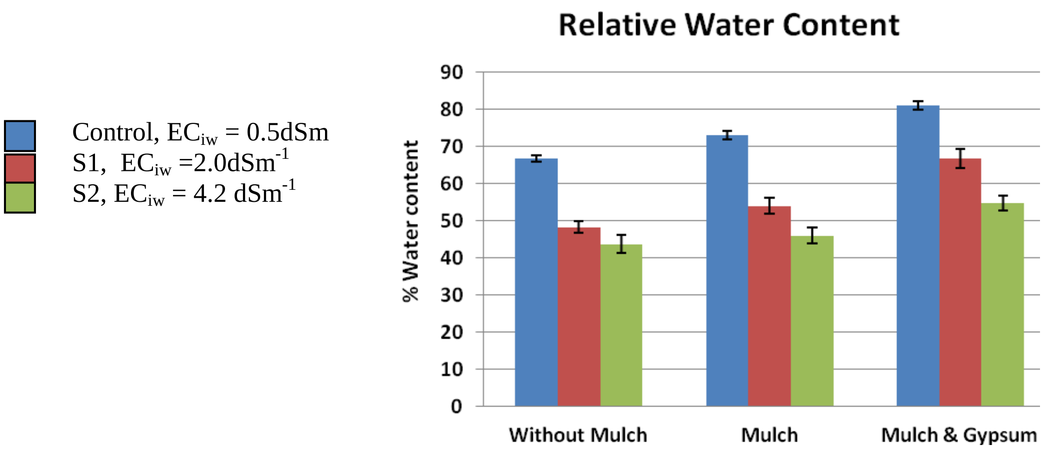

Fig. 2. RWC in leaf of Okra grown under sea salt concentrations at soil supplemented with mulch with or without gypsum.

Effect of Mulch on Leaf EL: Significant reduction in EL (F = 13.8619, P< 0.001) was found with application of mulch. Fig.1 revealed comparative greater reduction in EL under mulch& gypsum which may be due to reduction of soil salinity beneath the mulch (Table 1) or may accumulate certain amount of salts in mulch layer. Furthermore, mulch amended with gypsum is found more effective to reduce salinity hazards at greater extent under non-saline control and up to lesser extent with rising salinity in irrigation water and hence improve membrane stability by decreasing EL (Fig. 1). Interaction of mulch and salinity was also highly significant (P<0.0001, Table 2). Decrease EL in strawberry grown under wheat straw or black polythene has been reported by Kirnak *et al*., (2002). Tuna *et al*., (2007) reported supplemental $CaSO_4$ maintained membrane permeability by lowering in EL of tomato grown under 75 mM NaCl salinity. Khayyat *et al*., (2009) has also reported decrease EL in strawberry grown under salinity supplemented with calcium salts.

Table 2. **Two-way ANOVA for salinity and mulch treatments on EL of okra leaf.**

Electrolyte leakage

Source	SS	MS	Df	F	P
Mulch	277.53	138.768	2	13.08619	0.0002
Salinity	844.046	422.023	2	42.157	0.0000
Mulch x Interaction	1040.47	260.117	4	25.983	0.0000
Error	180.193	10.0107	18		
Total	2342.267		26		

DMRT (EL)

Mulch					Salinity				
Rank	**Treatment**	**Mean**	**N**	**NS-Ranges**	**Rank**	**Treatment**	**Mean**	**N**	**NS-Ranges**
1	3	24.189	9	a	Control	3	26.8	9	a
2	2	22.144	9	a	S1	2	22.7	9	b
3	1	16.6	9	b	S2	1	13.43	9	b
LSD0.05 = 3.135					LSD0.05 = 3.1335				

Effect of salinity on Leaf RWC: Results presented in Fig. 2 revealed decreased RWC of okra leaves under rising concentrations of sea salts in irrigation water. The reduction was highly significant (P< 0.0001) as presented in Table 3. Under saline conditions, plants face drought stress and hence their RWC is affected. Lowest RWC in leaf was observed at higher salinity (EC_{iw} 4.2 dSm^{-1}) as compare to non-saline control without any mulch treatments. Similar results have been reported by other scientists; Soliman *et al*., (2014) in five Apiaceae species; Baninasab and Baghbanha, (2013) in cucumber, Ahmad *et al*., (2012) in mustard cultivars and Salwa, (2010) in peanut plant.

Effect of Mulching on Leaf RWC: An increase in leaf RWC of okra grown under mulch alone and mixture of mulch and gypsum irrigated with different salinities is evident from present investigations (Fig. 2). Highest RWC was found under mulch with gypsum followed by mulch alone over without mulch control. ANOVA and DMRT test showed significant results for salinity (F = 25.794, P<0.0001) and mulch (F = 14.255, P = 0.001) treatment alone and their interaction (F = 28.689, P< 0.0001) as presented in Table 3.

Mulch + Gypsum > Mulch > Without Mulch

Organic mulches have been shown to be helpful in maintaining water content of soil and improve overall plant growth of hybrid tomato (Avinash F1 generation) under salinity (Saeed and Ahmad, 2009). Comparatively, higher RWC in wheat grown under rice husk mulch in semi-arid condition was reported by Chakraborty *et al*. (2008). However, Ranasinghe *et al*., (2003) reported that RWC of the leaves of coconut tree was unaffected by various mulches (coconut husk, coir dust, straw mulch and black polythene) during both wet and dry periods. RWC in plants is mainly dependant on the water status of the rhizosphere.

Table 3. Two-way ANOVA for salinity and mulch treatments on RWC of okra leaf.

Relative Water Content

Source	SS	Df	MS	F	P
Mulch	50.1556	2	250.778	14.255	0.002
Salinity	907.556	2	453.778	25.794	0.0000
Mulch x Interaction	2018.889	4	504.772	28.689	0.0000
Error	316.667	18	17.593		
Total	3744.667	26			

DMRT (RWC)

Mulch				
Rank	**Treatment**	**Mean**	**N**	**NS- Ranges**
1	1	69.222	9	a
2	2	62.667	9	b
3	3	58.7778	9	b
LSD0.05 = 4.1540				

Salinity				
Rank	**Treatment**	**Mean**	**N**	**NS- Ranges**
1	1	71.556	9	a
2	2	61.111	9	b
3	3	58.0	9	b
LSD0.05 = 4.1540				

Role of gypsum and calcium salts in alleviation of salinity was well documented. Divalent calcium from the gypsum replaces the monovalent sodium from the sodic soil resulting little increase in EC without detrimental effects on plant growth. It is also observed that sodium ion toxicity reduces by application of gypsum on sodic soil. Role of gypsum amended mulch in improving water status of the plant is evident from present study. Our results support Jafari *et al*., (2009) who observed the amelioration in salinity hazards in sorghum under 240 mM NaCl salinity. Zaman *et al*., (2005) reported that addition of 6 mM $CaSO_4$ results higher RWC in wheat under saline conditions.

Conclusion

It is concluded that organic mulch can be helpful in maintaining the RWC and membrane stability by reducing EL while irrigating with diluted sea water or saline solutions. Organic mulch (grass clippings + sawdust + cow dung) is more effective if supplemented with gypsum.

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