



Effect of water quality regimes, irrigation schedules, RSC levels and neutralizer on physiological and biochemical behaviour of fodder sorghum (*Sorghum bicolor*)

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ABSTRACT

Two independent experiments were carried out to study the physio-biochemical responses of fodder sorghum (*Sorghum bicolor* L.) under different irrigation water quality, irrigation schedules, RSC levels and neutralizer. Both these experiments were conducted during *kharif* season of 2013 in micro-plots of CSSRI, Karnal. First experiment was designed to evaluate the effect of different water quality irrigations scheduled at varying ID/CPE ratios. This experiment was laid out in factorial randomised block design (RBD) with four replications, consisting of nine treatment combinations of three irrigation water quality levels [Tube well water, cyclic use of Tube well water : Sewage water and Sewage water] and three irrigation schedules based on ID/CPE ratios (0.8, 1.0 and 1.2). Second experiment was carried out to assess the effect of RSC levels in irrigation water and their neutralization with gypsum and H₂SO₄. This experiment was conducted in randomised block design with four replications, consisted of five treatments of different RSC levels [control (No RSC), RSC – 5 meq l⁻¹, RSC–10 meq l⁻¹, RSC – 10 meq l⁻¹ (neutralized with gypsum), RSC – 10 meq l⁻¹ (neutralized with H₂SO₄)]. In first experiment, The sewage water recorded 19.18 and 11.73% higher *chlorophyll a* and total soluble protein at first cut and 9.56 and 11.92% at second cut compared to TW. The 1.2 ID/CPE ratio increased the *chlorophyll a* (26.5 and 16.5 %) and total soluble protein (22.9 and 25.4 %) compared to 0.8 ID/CPE ratio, at first and second cut, respectively. In second experiment, *chlorophyll a* and *b* was adversely affected with the application of RSC 10 meq l⁻¹ water with the maximum reduction by 17.71% at 1st cut and 19.62% at 2nd cut in case of *chlorophyll a* and 41.94% at 1st cut and 39.98% at 2nd cut for *chlorophyll b* compared to control (No RSC). Our results concluded that sewage water irrigation scheduled at 1.2 ID/CPE ratio proved superior and thus it may be adapted as a choice for getting higher green fodder yield with better physiological responses. From second experiment, it was inferred that higher RSC of irrigation water negatively affected the performance of fodder sorghum. It should be neutralized either by gypsum or sulphuric acid for obtaining better physiology as well as for fodder yield.

Key words: Chlorophyll, Forage sorghum, Proline, RSC, Sewage water, Total soluble protein

India is predominantly an agricultural country. The agricultural sector uses around 79% of the available freshwater supply and wastes one-half to four-fifth of the irrigation water. Due to increased population pressure the inter sectors (Agriculture, industry and domestic purpose) competition has been raised, this has resulted into declined availability of fresh water supplies to agriculture. In urban sector about 80 % of water supplied is returns back in

deteriorated qualities. It is estimated that 38000 MLD (Million litres per day) of wastewater is generated from the urban centre and out of which only 35% is treated (CPCB 2009). In addition to this around 13500 MLD wastewater is also generated from industrial sector. Besides being a source of irrigation water, these waste waters contain appreciable amounts of essential as well as toxic nutrients. Thus, reuse of domestic and industrial waste water in agriculture for irrigating crops is very crucial. Conjunctive use of waste water (sewage water) with good quality water and irrigation scheduling for using these water appears to be a viable option in increasing the availability of water supply in agriculture.

Saline/sodic water resources are more abundant than fresh water in arid/semiarid regions. In India, about 50 % of the groundwater is either marginal or poor in quality. Out of this, 37 % has high sodicity, 20% has high salinity and the remaining 43% has high salinity as well as sodicity.

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Particularly in Haryana, 55% of groundwater is in poor quality. Amongst the poor quality waters; 24, 30 and 46% are saline, sodic and saline-sodic, respectively (Yaduvanshi *et al.*, 2008). Continuous and indiscriminate use of this poor quality water (high in carbonates and bicarbonates) for irrigation poses a serious threat to the sustainability of agricultural production of this region (Minhas *et al.* 2007). The adverse effects of irrigation with sodic water can be mitigated by the application of amendments containing calcium such as gypsum or acidifying materials (H_2SO_4). Plants growing under salt stress have developed complex physiological and biochemical mechanisms to accumulate the compatible solutes (osmolytes) for maintaining the intracellular osmotic homeostasis (Gupta and Huang 2014; Roy *et al.* 2014). The accumulations of these osmolytes maintain turgor pressure and stabilize the various macromolecular structures against the salinity-induced physiological drought stress. Proline, an important osmolyte, contributes to osmotic adjustment by mitigating the ROS-induced oxidative damage (Gupta and Huang 2014). The increase in proline maintains the cellular osmolarity that provides necessary turgidity for cell expansion under stress conditions (Matysik *et al.* 2002). Salinity also disturbs photosynthesis mostly by decrease in chlorophyll content, leaf area, stomatal conductance, and reduced efficiency of photosystem II efficiency.

Sorghum (*Sorghum bicolor* L.) is suitable crop where maize, teosinte and pearl millet cannot be grown with alkali soil and alkaline irrigation water. It is cultivated in 100 countries, out of which 59 % of world sorghum area is in Africa, whereas, Asian countries occupy 25 % and Asia alone contributes 45% of world sorghum production (Mehmood *et al.* 2008). Sorghum is a fast-growing, warm weather annual plant that can provide plenty of feed in mid-summer during the lean period of fodder availability. Due to its excellent growing habit, high yield potential, and better nutritive value, it is much favoured by all farmers.

Therefore, present study was carried out with fodder sorghum crop in two separate experimental units. First experiment was designed to evaluate the effect of different water quality irrigation scheduled with varying ID (irrigation depth)/CPE (cumulative pan evaporation) ratios. The second experiment was conductively undertaken to assess the effect of RSC levels in irrigation water and their neutralization with gypsum and H_2SO_4 .

MATERIALS AND METHODS

Both studies were conducted during *kharif* season of 2013 in micro-plots at ICAR-Central Soil Salinity Research Institute, Karnal, Haryana, India. The climate of the experimental site was sub-tropical and rainfall was received mainly from July to September. Total 649.3 mm rainfall was received during the growing period. The soil of the experimental plots was slightly alkaline in reaction, low in organic carbon, low in available nitrogen, medium in available phosphorus and potassium.

Multi-cut forage sorghum hybrid Raseela (MFSH-4) of Mahyco Seed Company was used as test crop under

both the studies. The seeds of sorghum variety MFSH-4 were sown on 7th May by pora method with 8 rows in each micro-plot. Distance between the rows was kept 25 cm. The size of micro-plots was $2 \times 2 \text{ m}^2$. First cut was taken at 65 days after sowing (DAS) and the second cut was taken at 50 days after the first cut. Fertilizers (40:40:30 N: P_2O_5 : K_2O kg ha⁻¹) were applied through urea, diammonium phosphate (DAP) and muriate of potash (MOP). In view of best weed management, all the plots were manually weeded as per the requirement.

The first experiment was conducted in factorial randomized block design with four replications. Total nine treatment combinations with three irrigation water quality levels [tube well water (TW), cyclic use of tube well water (TW) : sewage water (SW) and sewage water (SW)] and three irrigation schedules based on ID (irrigation depth)/CPE (cumulative pan evaporation) ratios (0.8, 1.0 and 1.2). The second experiment was conducted in a randomised block design with four replications, consisted of 5 treatments of different RSC levels [RSC – nil (control), RSC – 5 meql⁻¹, RSC–10 meql⁻¹, RSC – 10 meql⁻¹ (neutralized 5 meql⁻¹ with gypsum), RSC – 10 meql⁻¹ (neutralized 5 meql⁻¹ with H_2SO_4)]. Analysis of variance two-way was applied to assess the effects of treatments by using Genstat 13 (GenStat® 13th Edition, VSN International limited Lawes Agricultural Trust, Rothamsted, UK) statistical software.

All the observations were recorded at 1st cut (65 DAS) and at 2nd cut (50 days after first cut). The Chlorophyll content was estimated according to the method of Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO). Calculations for different pigments were made as per the procedure described by Welburn (1994). Proline content was estimated in plant leaf samples using the method of Bates *et al.* (1973) and expressed as $\mu\text{g g}^{-1}$ FW. Total soluble proteins were determined by Bradford (1976) method and the total soluble protein was expressed as mg g⁻¹ DW. The method developed by Hogg and Ahlgren (1942) was used to determine the HCN content (mg/kg).

RESULTS AND DISCUSSION

Experiment-First

The data on *chlorophyll a* content as affected by irrigation water quality and different irrigation regimes scheduled at variable ID/CPE ratio are presented in Table 1. Across the water quality treatments, *chlorophyll a* increased with gradual switch over from TW to SW irrigation at both cuts. The highest *chlorophyll a* of sorghum recorded with SW irrigation at 1st cut ($32.06 \mu\text{g ml}^{-1}$) and at 2nd cut ($27.16 \mu\text{g ml}^{-1}$), whereas the lowest *chlorophyll a* recorded with TW irrigation at both cuts, *i.e.* [at 1st cut ($26.9 \mu\text{g ml}^{-1}$) and 2nd cut ($24.79 \mu\text{g ml}^{-1}$)]. The crop irrigated using SW recorded 19.18% higher *chlorophyll a* at first cut and 9.56 % at second cut compared to TW. Since N is the main constituent of all amino acids in proteins and lipids that act as structural compound of chloroplasts (Arisha and Bradishi 1999) thus presence of N in both waste water as well as in

Table 1 Effect of different water quality levels and different irrigation schedule of ID/CPE ratio on *chlorophyll a* of fodder sorghum

WQ/IS	Chlorophyll a (µg/ml)							
	TW		TW:SW		SW		AV	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
0.8	23.81	22.92	25.99	23.99	28.25	25.11	26.02	24.01
1	27.47	24.87	28.79	25.64	31.23	26.73	29.16	25.75
1.2	29.42	26.58	32.59	27.67	36.71	29.64	32.91	27.96
AV	26.9	24.79	29.12	25.77	32.06	27.16		
LSD (P=0.05)	2.577	1.332	WQ/IS		4.464	2.307	WQ*IS	

the form of inorganic fertilizers might have increased the total chlorophyll content, number of meristematic cells and growth leading to the formation of branches in addition to leaf expansion and number. *Chlorophyll a* increased with gradual increase in ID/CPE ratio from 0.8 to 1.2.

The highest *chlorophyll a* observed at ID/CPE ratio 1.2 at 1st and 2nd cut was 32.91 and 27.96, respectively. There was significant increase at 1.2 ID/CPE ratio as compared to 0.8 ID/CPE ratio at both cuts. Among different irrigation water treatments, the highest *chlorophyll b* was recorded with SW irrigation at 1st cut (16.63 µg ml⁻¹) and at 2nd cut (18.12 µg ml⁻¹), whereas the lowest *chlorophyll b* recorded with TW irrigation at 1st cut (13.62 µg ml⁻¹) and 2nd cut (12.91 µg ml⁻¹) (Table 2). The SW recorded 22.09% higher *chlorophyll b* at first cut, 40.35 % at 2nd cut compared to TW. *Chlorophyll b* content increased with gradual increase in ID/CPE ratio from 0.8 to 1.2. The highest *chlorophyll b* observed at ID/CPE ratio 1.2 at 1st and 2nd cut was 16.84 and 17.69 µg ml⁻¹, respectively. The lowest *chlorophyll b* observed at ID/CPE ratio 0.8 at 1st cut and 2nd cut was 13.1 and 13.32 µg ml⁻¹, respectively. There was significant

increase at 1.2 ID/CPE ratio as compared to 0.8 ID/CPE ratio at both cuts.

Proline content was varied by imposition of different water quality, ID/CPE ratios (Table 3). Maximum proline accumulation was achieved in TW irrigation at 1st cut (11.46 µg g⁻¹ of FW) and 2nd cut (10.50 µg g⁻¹ of FW). On the other hand, the lowest proline content was observed in SW treatment at 1st cut (10.70 µg g⁻¹ of FW) and 2nd cut (9.44 µg g⁻¹ of FW). The decrease in proline content in SW compared to TW was significant at 2nd cut. Proline content increased with a gradual decrease in ID/CPE ratio from 1.2 to 0.8. ID/CPE ratio, 0.8 ID/CPE ratio accumulated the highest proline content at 1st and 2nd cut (12.20 and 10.64 µg g⁻¹ of FW, respectively). The least proline was observed in ID/CPE ratio 1.2 at 1st and 2nd cut (10.14 and 9.4 µg g⁻¹ of FW, respectively).

Increased proline content observed with increasing moisture stress might be due to the transcriptional activation of the NADPH dependent PSC 5 (Babiychuk *et al.* 1996) and the concomitant increase in protease activity. This induces the breakdown of proteins under water stress conditions

Table 2 Effect of different levels of water quality and different irrigation schedule of ID/CPE ratio on *chlorophyll b* of fodder sorghum

WQ/IS	Chlorophyll b (µg/ml)							
	TW		TW:SW		SW		AV	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
0.8	11.60	11.78	12.70	12.45	15.05	15.74	13.12	13.32
1	13.93	13.14	14.39	14.15	16.71	18.16	15.01	15.15
1.2	15.31	13.81	17.05	18.81	18.15	20.45	16.84	17.69
AV	13.61	12.91	14.71	15.14	16.64	18.12		
LSD (P=0.05)	2.57	1.47	WQ		4.45	2.54	WQ*IS	

Table 3 Effect of different levels of water quality and different irrigation schedule of ID/CPE ratio on proline of fodder sorghum

WQ/IS	Proline (µg/g FW)							
	TW		TW:SW		SW		AV	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
0.8	12.57	11.04	12.03	10.68	11.99	10.21	12.20	10.64
1	11.29	10.41	10.88	9.87	10.31	9.30	10.83	9.86
1.2	10.51	10.06	10.10	9.32	9.80	8.82	10.14	9.40
AV	11.46	10.50	11.00	9.96	10.70	9.44		
LSD (P=0.05)	0.759	0.79	WQ/IS		1.315	1.369	WQ*IS	

Table 4 Effect of different levels of water quality and different irrigation schedule of ID/CPE ratio on total soluble protein of fodder sorghum

WQ/IS	Total soluble protein (mg/g DW)							
	TW		TW:SW		SW		AV	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
0.8	27.50	22.75	28.06	24.06	29.44	26.19	28.33	24.33
1	30.26	26.01	31.05	27.05	32.24	28.99	31.18	27.35
1.2	31.97	28.97	33.91	30.71	38.56	31.81	34.81	30.50
AV	29.91	25.91	31.01	27.27	33.41	29.00		
LSD (P=0.05)	1.289	0.616	WQ/IS		2.232	1.066	WQ*IS	

and consequently proline (Agarwal *et al.* 1995 and Jain *et al.* 1996). SW irrigation scheduled at 1.2 ID/CPE ratio resulted in the lowest proline content at 1st and 2nd cut with respective value of 9.80 and 8.82 $\mu\text{g g}^{-1}$.

The perusal of data on total soluble protein (TSP) content is depicted in Table 4. Mean maximum TSP content was shown by SW irrigation at 1st cut (33.42 mg g^{-1} of DW) and at 2nd cut (29.0 mg g^{-1} of DW). Whereas contrary to this, mean minimum TSP was recorded in TW irrigation (29.91 and 25.91 mg/g of DW at 1st and 2nd cut, respectively). The extent of superiority in SW irrigation was observed by 11.73 and 11.92 % over TW irrigation at 1st cut and 2nd cut, respectively. While concerning the effect of ID/CPE ratio TSP content increased with increased ID/CPE ratio. ID/CPE ratio of 1.2 showed maximum TSP (34.81 and 30.5 mg g^{-1} of DW at 1st and 2nd cut, respectively). ID/CPE ratio of 0.8 was noticed with minimum values of this attribute at both the recorded stage. The increment in TSP by employing ID/CPE ratio of 1.2 was 22.87 and 25.35 % at 1st and 2nd cut, respectively as compared to 0.8 ID/CPE. The maximum TSP content with values of 38.56 and 31.81 mg g^{-1} of DW at 1st and 2nd cutting, respectively was showed by SW irrigation scheduled at 1.2 ID/CPE.

Data on green fodder yield as exhibited in Fig 1. Among the water quality SW irrigation was noted superior as it resulted into maximum green fodder yield (31.31 and 21.16 t ha^{-1}) at 1st and 2nd cut, respectively. In comparison to TW irrigation, SW irrigation was observed 14.39 and 25.0 %

higher green biomass at 1st and 2nd cut, respectively. The presence of essential nutrients in SW might have resulted in higher yield. Nadia (2005) also recorded increased sorghum yield with the use of wastewater for irrigation. Within ID/CPE ratios, ID/CPE ratio of 1.2 was found superior with 31.41 and 21.64 t ha^{-1} green fodder yield at 1st and 2nd cut, respectively. The extent of increment by executing 1.2 in comparison to 0.8 was noted 15.3 and 20.7% at 1st and at 2nd cut, respectively. Better growth and physiological mechanism at this ratio might be possible reason for higher yield. Velu and Palaniswamy (2001) also reported similar results.

Experiment-Second

Data regarding the effect of sodic waters on chlorophyll content, total soluble protein, and proline are presented in Table 5. The *chlorophyll a* and *b* was adversely affected with increase in RSC level. *Chlorophyll a* and *b* content decreased significantly in RSC 10 compared to control. The percentage amount of reduction was observed 17.71 and 19.62% at 1st and 2nd cut, respectively in *chlorophyll a* by applying RSC 10 irrigation water compared to control. *Chlorophyll b* was reduced by 41.94 and 39.98% at 1st and at 2nd cut respectively by imposing RSC 10 in comparison to control. Application of gypsum and H_2SO_4 amendments did not brought any positive effect in *chlorophyll a* at 1st cut and in *chlorophyll b* at 2nd cut.

Irrigation in sorghum crop using RSC 10 water significantly reduced the total soluble protein compared to control. The highest values of 30.38 and 27.38 mg g^{-1} of DW TSP content at 1st and 2nd cut, respectively recorded in control while lowest (24.63 and 21.88 mg g^{-1} of DW at 1st and 2nd cut, respectively) TSP content was reported in RSC 10 water irrigation. The reduction in total soluble protein using RSC 10 water was 18.92% and 20.08% at 1st and 2nd cut respectively as compared to control.

Proline content increased with increasing levels of RSC in irrigation water. The highest proline content, *i.e.* 15.57 and 13.02 $\mu\text{g g}^{-1}$ of FW at 1st and 2nd cut, respectively was recorded with water of RSC 10, whereas the lowest proline was recorded in control, *i.e.* 11.59 at 1st cut and 10.6 $\mu\text{g g}^{-1}$ of FW at 2nd cut. Proline content was increased by 34.33 and 23.02% at 1st and 2nd cut, respectively by RSC water of 10 as compared to control. Proline content

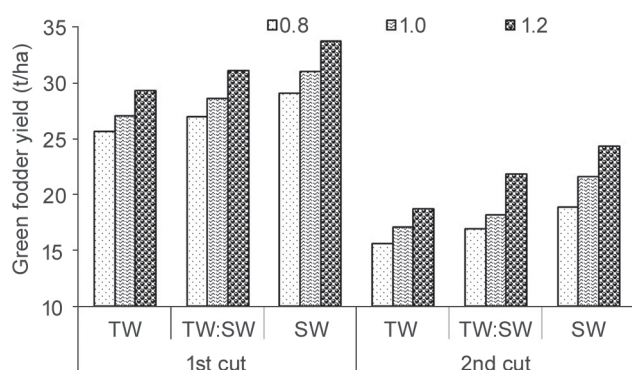


Fig 1 Green fodder yield (t/ha) as affected by different water quality levels and ID/CPE ratio.

Table 5 Effect of different RSC levels in irrigation water and their amendment with gypsum and H₂SO₄ on physiological parameters

Treatment	Chlorophyll <i>a</i> (µg/ml)		Chlorophyll <i>b</i> (µg/ml)		TSP (mg/g DW)		Proline (µg/g FW)	
	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut	1 st cut	2 nd cut
Control	25.06 ^b	23.8 ^b	12.42 ^b	11.83 ^b	30.38 ^b	27.38 ^b	11.59 ^a	10.6 ^a
RSC 5	23.64 ^{ab}	21.97 ^{ab}	10.06 ^{ab}	11.02 ^b	28.55 ^{ab}	24.04 ^{ab}	13.14 ^b	11.6 ^{ab}
RSC 10	20.62 ^a	19.13 ^a	7.21 ^a	7.10 ^a	24.63 ^a	21.88 ^a	15.57 ^c	13.04 ^c
RSC10+Gypsum	24.98 ^b	22.09 ^{ab}	11.22 ^{ab}	11.36 ^b	28.69 ^{ab}	24.33 ^{ab}	13.20 ^b	11.98 ^{bc}
RSC 10+H ₂ SO ₄	23.99 ^b	21.53 ^{ab}	10.72 ^{ab}	11.05 ^b	28.05 ^{ab}	22.91 ^{ab}	12.63 ^{ab}	11.16 ^{ab}
SEm±	0.95	1.28	1.42	0.85	1.38	1.6	0.33	0.36
LSD (P=0.05)	2.07	2.78	3.10	1.86	3.0	3.48	0.72	0.79

decreased significantly by imposing gypsum and H₂SO₄ amendments at 1st observation/cut whereas only H₂SO₄ was found effective in reducing proline at 2nd study stage. Proline is considered as an organic compatible osmolyte and has a protecting role in plant growth and productivity under salt or drought stress by reducing the production of free radicals and/or scavenging the free radicals (Dubey 1997 and Jain *et al.* 2001). Thus with higher RSC water more accumulation was observed.

HCN content increased with increasing concentrations of RSC in irrigation water and decreased by neutralization of RSC with gypsum and sulphuric acid amendments (Fig 3). The highest HCN content was recorded with RSC 10 irrigation water at 1st cut (139.31 mg kg⁻¹) and 2nd cut (115.8 mg kg⁻¹), whereas the lowest recorded in control (122.09 and 85.7 mg kg⁻¹ at 1st and 2nd cut, respectively). The increase in HCN content from application of RSC 10 irrigation water as compared to control was 14.10 and 35.12% at first and second cut, respectively.

It is apparent from Fig 4 that RSC 10 meq l⁻¹ water resulted in lowest green fodder yield at 1st cut (9.38 t ha⁻¹) and 2nd cut (6.7 t ha⁻¹), whereas with control treatment the highest green fodder yield (27.25 and 18.02 t ha⁻¹) was obtained. Green fodder yield was adversely affected with the application of RSC water with the maximum reduction by 65.57% at 1st cut and 62.9% at 2nd cut in RSC 10 water

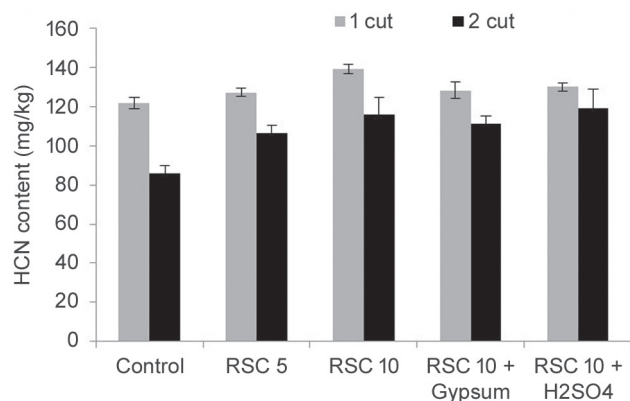
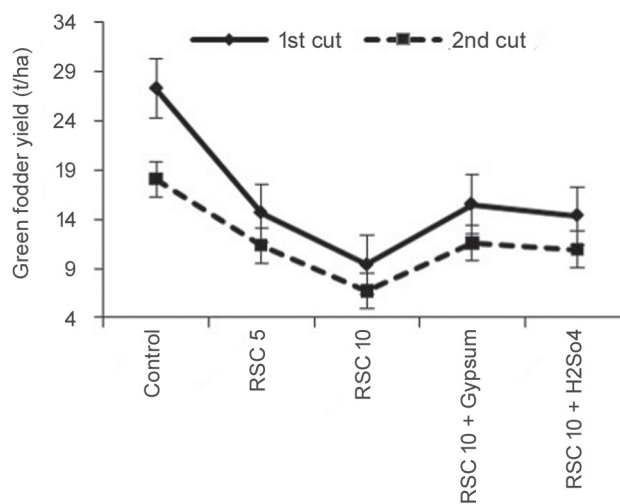


Fig 2 HCN content of sorghum as affected by different RSC levels in irrigation water.

Fig 3 Green fodder yield (t/ha) of sorghum as affected by different RSC levels in irrigation water and their amendment with gypsum and H₂SO₄.

but the reduction with RSC10+ gypsum was 43% at 1st cut and 35.8% at 2nd cut as compared to control.

Choudhary *et al.* (2001 and 2004) reported that irrigation with high RSC/Sodic water deteriorated soil properties and thus adversely affected yield and quality of tomato crop. Results obtained in our study are in agreement with the finding of Saquib *et al.* (2011), which indicated that gypsum significantly improved the fresh biomass of maize fodder compared to application of high EC-SAR-RSC water.

Conclusion

Our experimental findings indicated that the sewage water irrigation scheduled at 1.2 ID/CPE ratio proved superior in physiological responses and fodder yield compared to other water quality levels and ID/CPE ratio at both cutting. In second experiment, irrigation with increasing levels of RSC from 5 to 10 meq l⁻¹ adversely affected the performance of fodder sorghum. These adverse effects of sodic water irrigation on physiology and yield were decreased by using neutralizer (gypsum and sulphuric acid). Thus sewage water irrigation with 1.2 ID/CPE ratio and higher RSC of irrigation water with suitable neutralizer either

by gypsum or sulphuric acid could be used for obtaining better physiology as well as for fodder yield of sorghum.

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