

ORIGINAL ARTICLE

Effect of Cumulative application of Silicon and living mulch on the various traits of Sorghum (*Sorghum bicolor* L.) under drought Stress conditions

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ABSTRACT

The sorghum (*Sorghum bicolor* L.) and mung bean (*Vigna radiate* L.) are important forages in several arid and semi-arid climatic regions and are well adapted to environments with limited rainfall, high temperatures and low soil fertility. Application of Si suggested as an alternative approach to alleviate drought stress in crops. An experiment was conducted during the 2017 growing seasons to determine effect of the Effect of living mulch on nutritional quality forage Sorghum (*Sorghum bicolor* L.) due to drought stress and silicon fertilization condition. The experiment was arranged in a three-replicated split-plot factorial design with three irrigation regimes including optimum irrigation (when evaporation reached 60 mm, using evaporation pan class "A"), moderate drought stress (120 mm), and severe drought stress (180 mm) as main plots. while silicon levels i.e. 0 (Not used), (Spreading silica with a ratio of 3×1000 L in three steps and silicon irrigation fertilizer at a rate of 10 liters per hectare in three times) and living mulch (*Vigna radiate* L.) containing three levels (Not used living mulch and control weeds as control, without mulch and complete weed interference and use living mulch) was used as a sub factor. The result for the impact of drought stress mean and silica fertilizer on total wet weight showed that the most total wet weight 102.95 (Ton/ha) obtained from 60 mm evaporation from pan A. With application of silicon is with irrigation and foliar, CAT, SOD, RWC and proline content increased as compared to water deficient treatment and these results suggest that silicon application may be useful to improve the drought tolerance of sorghum through the enhancement of water uptake ability.

Key words: Drought stress, Silicon, Porolin, RWC & Sorghum

Received 02.05.2019

Revised 08.08.2019

Accepted 06.09.2019

How to cite this article:

E Rezaei, H Rajablarjani, P Kasraei, H Tohidi moghadam, F Ghoshchi: Effect of Cumulative application of Silicon and living mulch on the various traits of Sorghum (*Sorghum bicolor* L.) under drought Stress conditions. Adv. Biores., Vol 10 [5] September 2019:19-28.

INTRODUCTION

Agricultural productivity quantity is subject to evolving environmental constraints, particularly to drought and salinity due to their high magnitude of impact and wide distribution [20, 23]. Sorghum (*Sorghum bicolor* L.) is one of the most important crops of arid regions. Insecure water supply as a result of fluctuating precipitation and/or limited irrigation often causes a decrease in sorghum yield. Thus, it is important to enhance the drought tolerance of this economically important crop (Paterson, 2008). The evidence that silicon application decreases drought stress has been widely reported, but the mechanism it underlying remains unclear [32]. Silicon is considered an essential element in several crops enhancing growth and alleviating different biotic and abiotic stresses [33]. Si to culture solution could partially improve total, free, and bound water contents in both leaves and roots, which were all decreased under water stress and application of Si increased water potential (Ψ_w) and osmotic potential (Ψ_π) in both roots and leaves while maintained higher turgor pressure (Ψ_p), in comparison with the plants without Si application [34]. Silicon (Si) may be involved in metabolic, physiological, and/or structural activity in higher plants exposed to abiotic and biotic stresses. This has not yet been determined due to the absence of direct evidence that it is part of the molecule of an essential plant constituent or metabolite [1]. The silicon application may be useful to improve the drought tolerance of sorghum via the enhancement of

water uptake ability and ameliorated the decrease in dry weight under drought stress conditions [31]. And the silicon-applied sorghum could extract a larger amount of water from drier soil and maintain a higher stomatal conductance [31]. Proline was increased by drought stress and decreased by silicon [33]. Living mulches are vegetative covers that can be grown in association with row crops to reduce soil erosion, improve traffic ability and suppress weeds [4, 7]. Cover crops are also known as “green manures,” “catch crops,” or “living mulch.” Green manure cover crops are usually legumes that fix N and are grown to provide N to the following cash crop [3]. The objective of this study is to clarify the effected of silicon application and living mulch on cellular antioxidative defense strategies and lipid peroxidation and sorghum forage under drought stress.

MATERIAL AND METHODS

A field experiment was conducted at the experimental farm of Islamic Azad University, Varamin Branch, Tehran, Iran (Latitude 35.325241, longitude 51.647198) during the 2017 growing seasons. The area has an arid to semi-arid climate with 38-year average annual precipitation of 251 mm, annual average temperature of 13.5°C, annual average soil temperature of 14.5°C, and total annual class “A” pan evaporation of 2207 mm (Fig).

The average monthly precipitation and temperature obtained from the Varamin Synoptic Meteorology Station, located at the experimental farm, are presented in Fig 1.

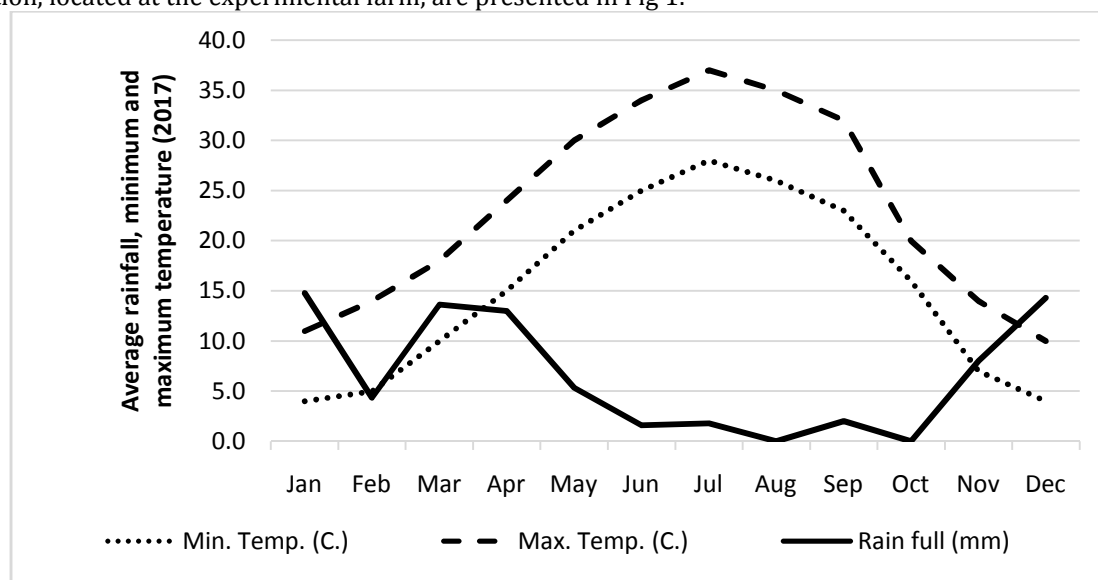


Fig 1. Average rainfall, minimum and maximum temperature on a standard week basis in the 2017 growing seasons

Table 1. Based on evaluated on trial treatments.

Table 1. Treatments			
Drf.	Main factors	Sub factors	
	Irrigation intervals (evaporation from class A evaporation pan)	Potassium silicate K2O -10%, SiO2 - 20%	Living mulch mung bean (<i>Vigna radiate</i> L.)
1	60 mm	Control (not used)	Control (not used)
2	120 mm	Fertilizer at a rate of 10 *3 liters per hectare	Without living mulch and complete weed interference
3	180 mm	Spray 3×1000 L in three steps	Use living mulch

The experimental site was plowed by moldboard plow, harrowed and divided into three blocks, each contained 27 plots. Each subplot was 2.5 m wide and 5 m long and consisted of four rows of sorghum which were 0.6 m wide and 5 m long. A gap of 1.5 meters was considered between adjacent main plots and the distance between replications was 3 m Plots were seeded on the 10 th and 5 th of June in 2017 and 2018, respectively, at a row spacing of 60 cm and four rows of mung bean (*Vigna radiate* L.) were

planted as living mulch. Prior to seeding, soil samples from each plot were taken from the top 30 cm of soil to test its background nutritional level. Selected chemical properties of soil are presented in (Table 2).

Table 2. Selected properties of the top soil (0–30 cm) at the experimental site.

Depth (cm)	Ds.m (Ec)	pH	Clay (%)	SALT (%)	SAND (%)	TEXTURE	
0-30	3.8	7.3	53	22	25	Clay	

P (ppm)	K (ppm)	N (ppm)	OC (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
10	210	1.1	0.8	1.5	1.1	2.5	2.2

After planting, plots were irrigated equally to facilitate seed germination. The hand weeding in control treatment was done twice (20 and 45 days after crop emergence). Irrigation regimes were applied when plants were completely established and plots were irrigated anytime evaporation reached the considered amount for each irrigation level (60, 120, and 180 mm evaporation from the surface of the evaporation pan). To control and calculate the amount of water, drip irrigation was performed. The diameter of tape was 16 mm, emitter spacing 15 cm, and flow rate 2 lit. h⁻¹. Numbers of irrigations were 21, 13, and 8 for Ir60, Ir120, and Ir180, respectively (Table 3).

Table 3. Date of irrigation for each irrigation regime during the 2017		
2017		
10-Jun	10-Jun	10-Jun
16-Jun	16-Jun	16-Jun
23-Jun	27-Jun	01-Jul
29-Jun	07-Jul	16-Jul
05-Jul	18-Jul	31-Jul
10-Jul	29-Jul	15-Aug
15-Jul	11-Aug	30-Aug
20-Jul	23-Aug	14-Sep
26-Jul	01-Sep	29-Sep
02-Aug	11-Sep	-
08-Aug	21-Sep	-
14-Aug	04-Oct	-
21-Aug	-	-
29-Aug	-	-
05-Sep	-	-
13-Sep	-	-
21-Sep	-	-
28-Sep	-	-
07-Oct	-	-
-	-	-

Numbers of irrigations were 19, 12, and 9 for Ir60, Ir120, and Ir180, respectively. Each plot received an approximate amount of 0.85 m³ of water in each irrigation and the end of each plot was blocked to control the volume of water. In the first year of the experiment (2017), the first and second forage cut took place on Aug 11 th, and Oct 8th, while it was Aug 5 th and Oct 6 th for the second year (2017), respectively. In this experiment, the traits relative water content (RWC), Superoxide dismutase (SOD), Catalase (CAT), malondialdehyde (MAD), proline and total wet weight were investigated. To measure the agronomic traits with respect to margins, 15 plants were randomly selected within each plot and plant height was measured. In order to measure the total wet weight function in full flowering stage of sorghum 1 square meter in the middle of each plot were cut to 2 cm above the soil surface and separated by hand into sorghum in July 2017 and reported in terms of tons per hectare. CAT was measured from young and developed leaves and then with the method according to the method Upadhyaya *et al.*, [21] in a reaction mixture containing 46mM potassium phosphate buffer, pH 7.0, 0.03% H₂O₂ and an enzyme extract. The decomposition of H₂O₂ was followed at 240 nm (1 EU / 1 mmol H₂O₂ decomposed in 1 min). Based on

method Beauchamp & Fridovich [20] SOD activity was measured by the photochemical method, one unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin in the light. The reaction mixture contained 45mM potassium phosphate buffer, pH 7.0, containing 0.1mM EDTA and 13mM methionine, 0.17mM NBT in ethanol, 0.007mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15min. The relative water content (RWC) concluded by (fresh weight – dry weight)/(turgid weight – dry weight) × 100 [22]. To evaluate the proline content in sorghum leaves according to by the acid-ninhydrin method of Bates *et al.* [23] was performed. The level of malondialdehyde (MAD) in the leaf tissue was measured in terms of MAD content determined by the TBA reaction, with minor modifications to the method of Heath and Packer [22]. To supply required nutritious, triple superphosphate fertilizer has been used between the first and the second disk interval (80 kg/ha). Accordingly, the required nitrogen has been supplied from the urea source (150 kg/ha). To precisely apply fertilizers, grooves were initially made in the irrigation furrows in each plot then the fertilizers was placed evenly inside the grooves, covered by soil and irrigated immediately.

RESULT AND DISCUSSION

Table 1. Analysis of variance for various studied traits of sorghum on proline, RWC, SOD, CAT, MAD and total wet weight

Source	Df.	M.s.					
		Prolin	RWC	SOD	CAT	MAD	Total wet weight
Rep.	2	0.682n.s	138.506**	40.974**	1.741**	124.847**	178.01*
Drought stress (D)	2	194.456**	1610.542**	307.417**	17.031**	2148.956**	19966.43**
Error a	4	2.0243*	126.905**	9.774**	0.432*	98.268**	73.66n.s
Living mulch (L)	2	0.076n.s	0.340n.s	1.1520n.s	0.041n.s	8.436n.s	395.043**
Silicon (S)	2	6.223**	774.929**	15.826**	1.929**	344.354**	1698.02*
D*L	4	0.105n.s	11.639n.s	0.500n.s	0.0949n.s	1.4373n.s	293.32**
D*S	4	2.067*	223.269**	4.139**	0.389*	51.761*	139.63*
S*L	4	0.0159n.s	20.342n.s	0.148n.s	0.037n.s	2.277n.s	30.82n.s
D*S*L	8	0.070n.s	4.507n.s	0.293n.s	0.057n.s	2.503n.s	10.17n.s
Total	48	0.541	22.432	1.851	0.121	15.291	49.8
Cv (%)		11.64	6.31	16.52	16.03	11.74	9.55

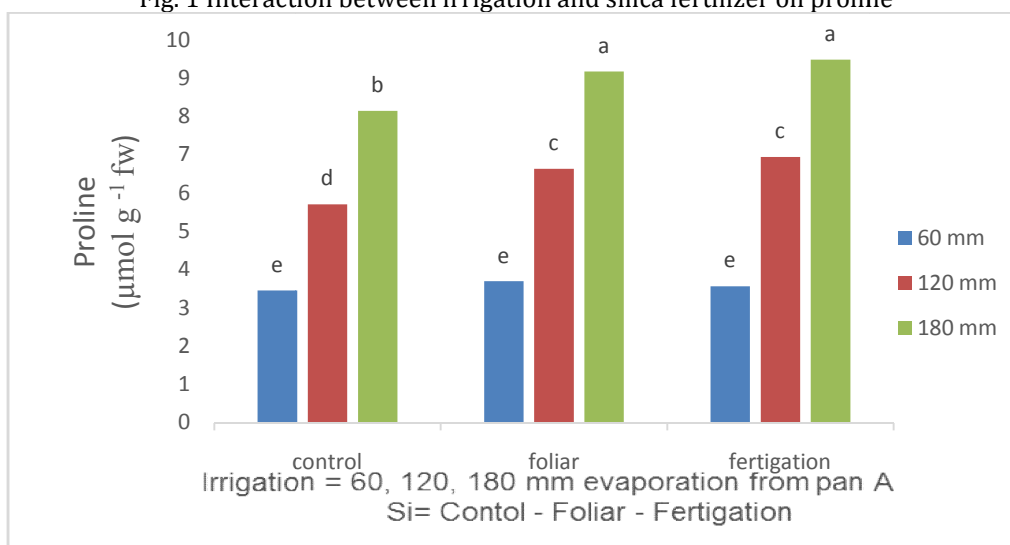
n.s. = Non-significant * = Significant at 5% level ** = significant at %1 level
Y= Year D= Drought - L= Living mulch - S= Silicon

Results of analysis of variance indicated the significant impact between drought stress and silica at 1% the simple effect of living mulch was significant at 1% on Total wet yield. The interaction effects between drought stress and living mulch were significant at 1% besides the total wet yield. The interaction effect between the irrigation period and silica fertilizer were significant at 1% for all traits. Other simple and interaction traits was not significant.

Proline

The most proline content resulted from 180 mm evaporation from pan A and application of silica 9.49($\mu\text{mol g}^{-1} \text{fw}$) and in normal irrigation condition (60 mm evaporation from pan A) the lowest proline was estimated and all silica fertilizer treatments were in same statistical group. This result showed that application silica in normal irrigation were not any effect on proline content (Fig. 1). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress [26]. The results showed that with an increase in the Intensity of drought stress on wheat cultivars, there was a decrease in relative water content, total chlorophyll content and increased proline content [27]. Mauad *et al.*, [25] reporter that under water stress conditions, silicon fertilization reduces the proline content of upland rice plants in both the vegetative and reproductive phases and increases peroxidase activity in the reproductive phase, which could be indicative of stress tolerance.

Fig. 1 Interaction between irrigation and silica fertilizer on proline

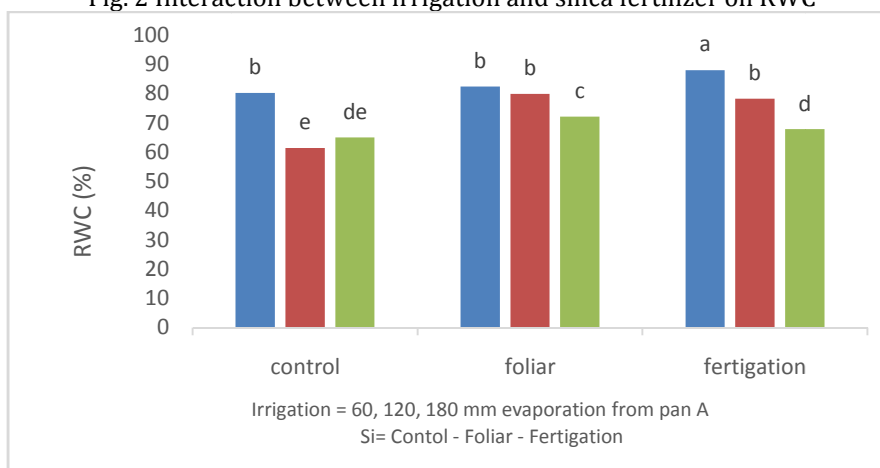


Different letters on bars corresponding to the same response variable, Interaction of drought stress and silica fertilizer on proline according to LSD test ($P < 0.05$).

RWC

In relation to changes of RWC, the result shows that RWC recorded the highest values 88.08 % in 60 mm evaporation application silica fertigation and the lowest amount of RWC were obtained from 180 mm evaporation from plan a treatment and no use of silica fertilizer with a mean of 61.46% (Fig. 1). Bukhari *et al.* [13] reported that drought stress significantly reduced the water relations in wheat as compared to normal conditions, however, exogenous Si supply was found to be effective in maintenance of turgidity. Similar results was obtained by Gunes *et al.* [17] who reported that the silicon applied to the soil at 100 mg kg⁻¹ significantly increased Si concentrations of the cultivars and counteracted the deleterious effects of drought in 5 of the ten chickpea cultivars by increasing their RWC. In another reported the relative leaf water content (RWC), which was the main factor resulting in reduced growth in response to drought, increased 19.0% and 30.0% with Si application under drought and drought+UV-B stresses, respectively [1].

Fig. 2 Interaction between irrigation and silica fertilizer on RWC



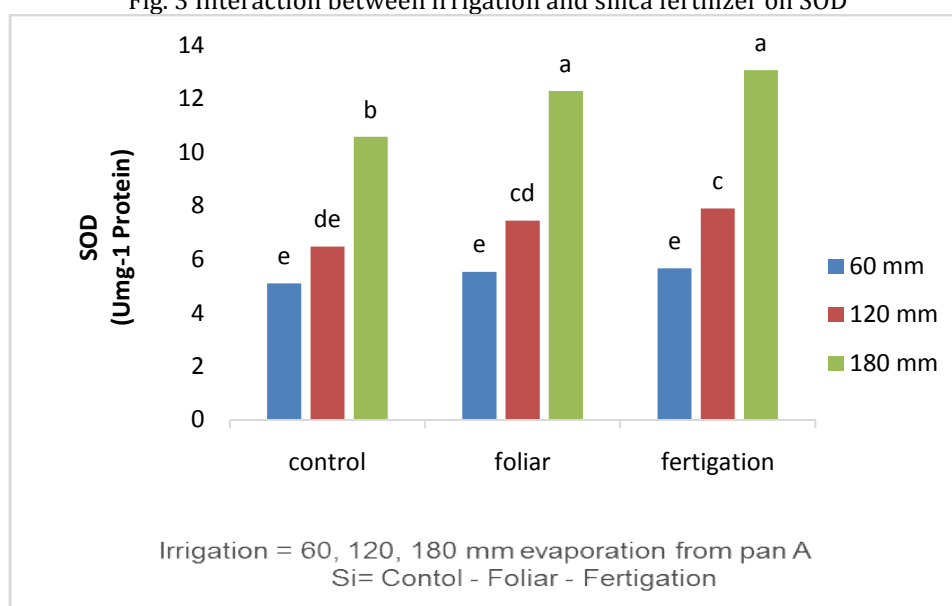
Different letters on bars corresponding to the same response variable, Interaction of drought and stress silica fertilizer on RWC according to LSD test ($P < 0.05$).

SOD

the result showed that the water stress cause increased SOD but, Total SOD was observed to more increased due to Silica supplementation. According to the Figure 3 results of the mean interactions of irrigation and silica fertilizer on the SOD showed that the irrigation from 180 mm evaporation from plan A treatment and application of silica in fertigation and silica foliar with an average of 13.07 and 12.29 respectively.(Umg⁻¹ Protein) and the lowest amount of SOD were obtained from 60 mm evaporation from plan A No use of silica fertilizer with a mean of 5.11(Umg⁻¹ Protein). Ahmad & Haddad, (2011) reported

the indicated that Si partially offset the negative impacts of drought stress increasing the tolerance of wheat by rising Pro and GB accumulation and soluble protein content. Compared with the plants treated with drought, applied Si significantly enhanced the activities of SOD, CAT, APX and POD. Gong *et al.*, [17] reported that in the stress conditions, plants growing in Si-applied soil could maintain higher relative water content (RWC), water potential and leaf area compared to those without Si applied. In connection to these results Gunes *et al.*, [16] pointed that the SOD and CAT activities of the cultivars were decreased by drought. Depending on cultivars, the CAT activity was decreased, and increased or unchanged in response to applied Si, while the SOD activity of the cultivars increased or unchanged by Si and The non-enzymatic antioxidant activity of the cultivars was also increased by Si. Inal *et al.*, [2] reported that the compared with control plants, the activities of AA, SOD, CAT, and APX in B stressed plants grown without Si decreased, and application of Si increased their activities under stress.

Fig. 3 Interaction between irrigation and silica fertilizer on SOD



Different letters on bars corresponding to the same response variable, Interaction of drought and stress silica fertilizer on SOD according to LSD test ($P < 0.05$).

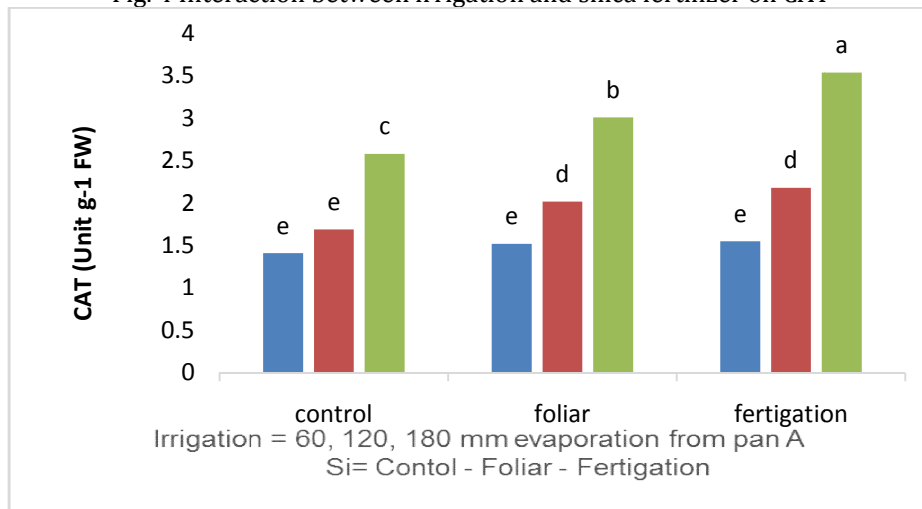
CAT

Changes in the activities of CAT enzyme under water stress have been reported [30]. The results of the mean interactions of irrigation and silica fertilizer on the percentage of CAT showed that the irrigation from 180 mm evaporation from plan A treatment and application of silica in fertigation with an average of 3.54 (Unit g^{-1} FW) and the lowest amount of CAT were obtained from 60 mm evaporation from plan A and no use of silica fertilizer with a mean of 1.41 (Unit g^{-1} FW), also in three traits of silica on 60 mm evaporation were in the same statistical levels (Fig. 4). In related to these study the water stress caused a marked decrease in water relations and uptake of phosphorous, potassium, magnesium and zinc in plants. The Si application significantly enhanced the plants ability to withstand water deficit conditions through increased Si uptake and improved activity of ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) [13]. Siddiqui *et al.*, [15] reported that the increase in plant germination and growth characteristics through application of SiO₂ might reflect a reduction in oxidative damage as a result of the expression of antioxidant enzymes, such as catalase, peroxidase, superoxide dismutase, glutathione reductase, and ascorbate peroxidase.

MDA

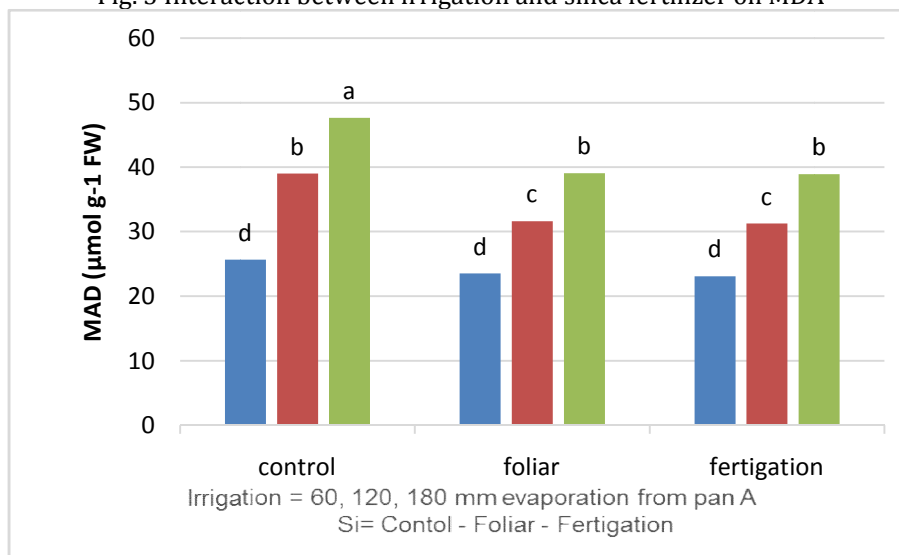
The results of the of the mean interactions of irrigation and silica fertilizer on the percentage of MDA showed that the irrigation from 60 mm evaporation from plan A and application of silica in fertigation 180 mm evaporation from plan A treatment and no use of silica fertilizer with an average of 47.64 ($\mu\text{mol } g^{-1}$ FW) and the lowest amount of MDA were obtained from 180 mm evaporation from plan A treatment and no use of silica fertilizer with a mean of 38.93 ($\mu\text{mol } g^{-1}$ FW) (Fig. 5). These result agree with the finding of Moussa [12] who reported that Si has been shown to decrease the concentration of malondialdehyde (MDA), the end product of lipid peroxidation, in salt-stressed barley maize. According the Application Si significantly enhanced the enzyme activities in roots of salt-stressed plants compared to Si-deprived salt treatments [34].

Fig. 4 Interaction between irrigation and silica fertilizer on CAT



Different letters on bars corresponding to the same response variable, Interaction of drought and stress silica fertilizer on CAT according to LSD test (P < 0.05).

Fig. 5 Interaction between irrigation and silica fertilizer on MDA

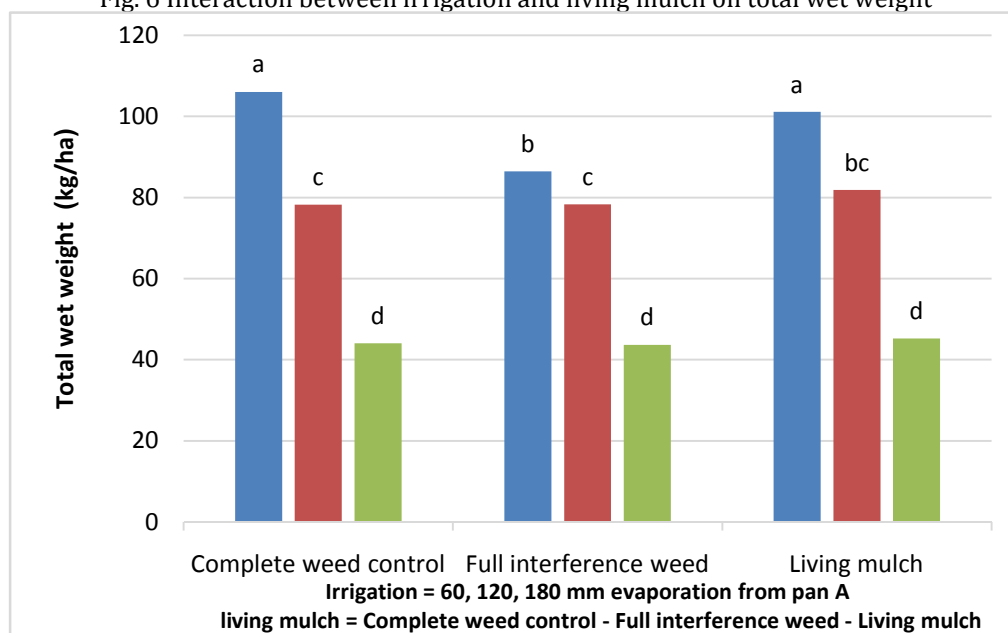


Different letters on bars corresponding to the same response variable, Interaction of drought and stress silica fertilizer on MDA according to LSD test (P < 0.05).

Total wet weight

The most yield of total wet weight achieved from 60 mm evaporation from pan A with application of living mulch (101.1 ton/he) and complete weed control(106.04 ton/he) and lowest total wet weight achieved from 180mm evaporation from pan A along with complete mixture of weed (43.69 ton/ha) (Table 6). In relation to these results Prasanthi [6] showed that the highest total green and dry fodder yields (50.1 and 14.2 t ha⁻¹) were recorded in maize in pairs + cowpea intercropping and the lowest, green and dry fodder were observed in sole pillipesara treatment (14.7 and 3.4 t ha⁻¹). Intercropping sorghum with guinea grass sown simultaneously yielded the highest yield per ha, which was 2.4 times greater than the revenue achieved by yield sorghum only [5].

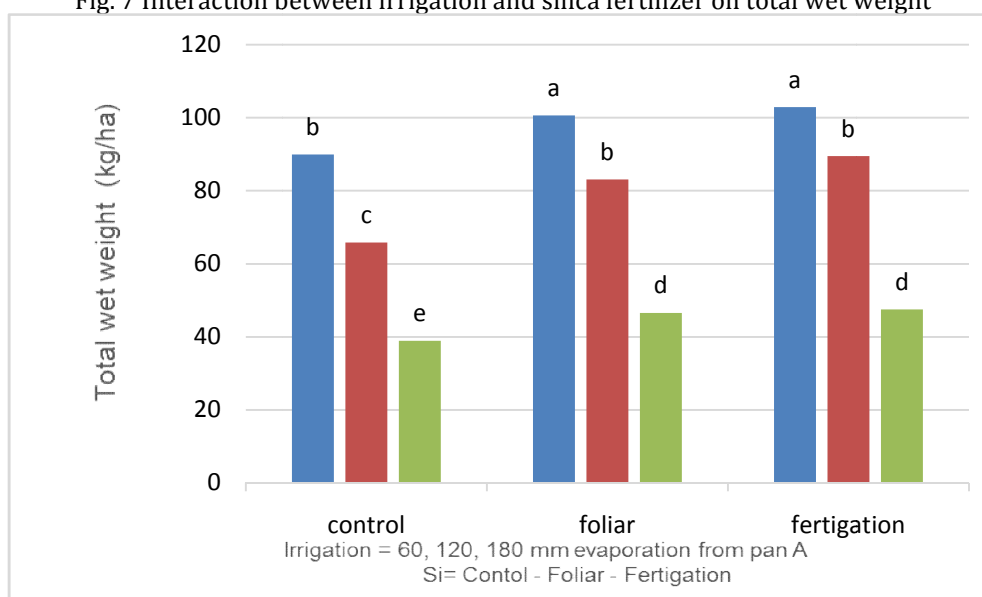
Fig. 6 Interaction between irrigation and living mulch on total wet weight



Different letters on bars corresponding to the same response variable, Interaction of drought and stress in living mulch on total wet weight according to LSD test ($P < 0.05$).

Moreover, The result for the impact of drought stress mean and silica fertilizer on total wet weight showed that the most total wet weight 102.95(Ton/ha) obtained from 60 mm evaporation from pan A and water application of silica fertilizer and 100.64 (Ton/ha) from foliar application while the lowest weight 38.95(Ton/ha) achieved from 180 mm evaporation from pan A without application of silica fertilizer (Table. 7). Ahmed *et al.*, [9] reported that when silicon concentration is applied with irrigation LAI, SPAD, LDW, TDW, net assimilation rate, relative growth rate, leaf area ratio and water use efficiency (WUE) increased by 30, 31, 40, 30, 28, 30, 27, 35, 32, 30 and 36% respectively as compared to water deficient treatment and these results suggest that silicon application may be useful to improve the drought tolerance of sorghum through the enhancement of water uptake ability. In relation to these results Saud *et al.*, [10] showed that the water stress was found to decrease the photosynthesis, transpiration rate, stomatal conductance, leaf water content, relative growth rate, water use efficiency, and turf quality, but to increase in the root/shoot and leaf carbon/nitrogen ratio and Such physiological interferences, disturbances in plant water relations, and visually noticeable growth reductions in Kentucky bluegrass were significantly alleviated by the addition of Si after water stress. As these obtained by Habibi & Hajiboland, [11] that the silicon treatment significantly increased plant dry weight and relative water content under drought stress and the application of Si for drought-stressed plants improved the maximum quantum yield of PSII. A reduction in the net assimilation rate due to drought stress was alleviated by Si application, accompanied by an increase in stomatal conductance.

Fig. 7 Interaction between irrigation and silica fertilizer on total wet weight



Different letters on bars corresponding to the same response variable, Interaction of drought and stress silica fertilizer on total wet weight according to LSD test ($P < 0.05$).

CONCLUSION

Results finally indicated that the effect of drought stress can be alleviated by silica in sorghum and using of living mulch improved the total wet weight of forage. The most quantity of the forage obtained from the irrigation treatment of 60 mm evaporation from plan A along with application of living mulch. Furthermore, Silica positively influence the growth and physiological parameter of sorghum. The application of silica fertilizer in drought stress not only increased the total wet weight, but also enhanced the RWC, SOD, CAT and proline content decrease MAD. According to this study, Increment in drought tolerance is associated with anti-oxidative enzyme activity, allowing sorghum plants to cope better with drought stress and the result showed that the living mulch has not been any effect on enzymes and biomarkers. However, further research is needed to efficiently improve the yield of forage within different areas.

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