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RESEARCH PAPER

Bio-fortification of calcium, zinc and iron improves yield and quality of forage sorghum (*Sorghum bicolor* L.)

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Key Message: The experiment provides a clear image that combined application of Ca at 3%, Zn at 2% & Fe at 1% improved not only the yield but also the quality of forage sorghum.

Abstract: Sorghum (*Sorghum bicolor* L.) is an important summer fodder crop. It is a quick growing grass which requires low inputs. Deficiency of major micronutrients {calcium (Ca), zinc (Zn) and iron (Fe)} is common in sorghum which creates some health problems in livestock. Therefore, the study was conducted during summer 2018 at Agronomic Research Farm, College of Agriculture, University of Sargodha Pakistan to evaluate the impact of calcium (Ca), zinc (Zn) and iron (Fe) bio-fortification on yield and quality of forage sorghum under field conditions. Experiment was laid out in randomized complete block design (RCBD) with 4 replications, the treatments were comprised as Control (no spray), T_1: Ca at 3%, T_2: Zn at 2%, T_3: Fe at 1%, T_4: Ca at 3% + Zn at 2%, T_5: Ca at 3% + Fe at 1%, T_6: Zn at 2% + Fe at 1% and T_7: Ca at 3% + Zn at 2% + Fe at 1%. Results confirmed that combined application of nutrients (Ca at 3% + Zn at 2%+ Fe at 1%) increased plant height (30.0%), number of leaves (53.6%), stem diameter (48.6%), leaf area per plant (77.2%), fresh biomass (48.2%), dry matter yield (120.8%), dry matter contents (49.1%), crude proteins (78.6%) and ash contents (120.8%) as compared to control (no spray). However, maximum NDF and ADF contents were obtained from control treatment. Ca and Fe contents were increased after the application of Ca at 3% and Fe at 1%. Although Ca at 3%, Zn at 2% & Fe at 1% gave highest plant contents of Zn. On the basis of our results, it is concluded that combined application of Ca at 3%, Zn at 2% & Fe at 1% improved not only the yield but also the quality of forage sorghum.

Keywords: Bio-fortification, Forage quality, Micronutrients, Sorghum, Yield

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Introduction

Sorghum (*Sorghum bicolor* L.) is a member of the grass family (Poaceae). It is a tall annual plant with an adventitious root system. It is drought and heat resistant fodder crop with fairly high biomass (Iqbal & Iqbal, 2015). In Pakistan, the local name of sorghum is *Jowar* which is an important fodder and grain crop (Iqbal et al., 2010). Globally, sorghum ranked 5th among all cereal crops with production of 60 million tons and Pakistan produced 0.22 million tons annually (Agricultural Statistics of Pakistan, 2016). Sorghum as a fodder crop is considered as essential forage for the livestock having ability to maintain good health of livestock. After pearl millet, sorghum is an economical source of energy and micronutrients. Approximately 35% of sorghum is grown for humans, and rest is mainly utilized as alcohol extraction, industrial products and animal feed (Gerrano et al., 2014). However, numerous factors affect its quality and yield such as selection of suitable varieties, inadequate application of nutrients, improper harvesting stage and existence of high hydrocyanic acid contents (Chattha et al., 2017). Ca, Zn and Fe contents are also low in sorghum and their deficiency normally causes health problems in livestock (Abdelhalim et al., 2019). Nutrition of all plants depends on the availability and uptake of macro and micro nutrients from soil. Calcium is an important nutrient for plant cell wall which maintains the structure of plant tissues (Hepler, 2005; Mousavi et al., 2012). Calcium lies in its ability to serve as a second messenger in a variety of processes ranging from root or pollen tube growth and fertilization (Thor, 2019) and its deficiency stops the development of new root and shoot tissues (El-Habbasha & Ibrahim, 2015).

Zinc is a basic plant micronutrient which performs vital roles in plants such as synthesis of protein, carbohydrate metabolism and protection against heat stress (Tsonev & Cebola Lidon, 2012; Cabot et al., 2019). It also plays a significant role in biomass production (Mousavi et al., 2013). Zn deficiency affects the crop plant in many ways i.e. stunting growth, sterility of spikelet, extending crop maturity period, and poor quality of harvested products (Hafeez et al., 2013). Mousavi et al. (2012) investigated that Zn and Fe have positive effects on the biomass, biomass components and composition of plants. Zn application improves kernel number and weight in maize (Liu, 2020). Iron is an essential micronutrient and has a vital role in many biological processes i.e. chlorophyll biosynthesis and photosynthesis being a component of the photosynthetic apparatus (Lopez-Millan et al., 2016) and its deficiency cause yellowing of the upper leaves, stunted growth and interveinal chlorosis (Rout & Sahoo, 2015). Singh et al. (2018); Vanlalruati et al. (2019) investigated that Fe and Zn application can be a good agricultural practice to enhance yield, protein content and the Zn and Fe concentration in grain. It also gives maximum no. of flowers, yield and weight of flowers rather than control. Fe and Zn deficiency in sorghum grain can be removed through bio fortification and it is a sustainable solution to their deficiencies (Kumar et al., 2015).

Profitable and well-organized livestock industry required sufficient quantity of good quality forage. Our methods of forage production are old and conventional which do not fulfil fodder requirements both with respect to quality and quantity, which results in animals starving while there are many methods for increasing supply of fodder. Recent studies showed that foliar application of a minute concentration of nutrients, especially Ca, Zn and Fe significantly increased the crop yield (Xia et al., 2019). Whereas, study regarding bio-fortification of these nutrients on yield and yield components of fodder sorghum is very limited. Therefore, the aim of this study is to evaluate the effects of bio fortification (foliar spray) of Ca, Zn and Fe on the forage sorghum.

Materials and Methods

Site and soil description

Experiment was carried to investigate the influence of bio-fortification of Ca, Zn and Fe on forage sorghum under field conditions during summer 2018 at Agronomic Research Farm, College of Agriculture, University of Sargodha Pakistan. Experimental soil analysis was performed before sowing the crop. Physicochemical soil properties are expressed in Table 1.

Table 1 Physicochemical analysis of experimental soil

Depth (cm)	Texture	Electrical conductivity (dS/m)	Saturation (%)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	Organic matter (%)	pH
0-15	Loam	0.0037	38	12.4	160	0.90	7.5

Experimental detail and crop husbandry

The trial was designed in randomized complete block design with four replications and the net plot size was 2.4 m × 5 m. The experiment was comprised of following treatments: No spray (control), T_1: Ca at 3%, T_2: Zn at 2%, T_3: Fe at 1%, T_4: Ca at 3% + Zn at 2%, T_5: Ca at 3% + Fe at 1%, T_6: Zn at 2% + Fe at 1% and T_7: Ca at 3% + Zn at 2% + Fe at 1%. Seeds of forage sorghum (Variety: Sargodha 2011) were sown at the recommended depths (3-4 cm) with single row manual hand drill in a fine seedbed prepared in proper moisture condition. Nitrogen (N) and phosphorous (P) were applied @ 90 kg ha^{-1} and 60 kg ha^{-1}, respectively. All other agronomic practices such as pre-sowing irrigation, hoeing, fertilizer application, seed rate and plant protection strategies were kept similar for the whole experiment. Spray of different doses of Ca, Zn and Fe was done after 25 days of germination (4 leaf stage of sorghum). Sources of Ca, Zn and Fe were $CaCl_2.2H_2O$, $ZnSO_4.H_2O$ and Fe_2SO_4, respectively. The crop was harvested with hand sickle after 75 days of sowing (DAS) when it reached almost 50% heading. Different parameters regarding yield and quality components of forage were recorded at harvest.

Data recorded

Crop was observed for different forage yield and quality parameters including plant height (cm), number of leaves per plant, stem diameter (cm), leaf area per plant (cm^2), fresh forage yield (t/ha), dry matter yield (t/ha), crude protein (%), neutral detergent fibre (%), acid detergent fibre contents (%), ash contents (%), plant calcium contents (%), plant zinc contents (%) and plant iron contents (%) by using standard procedures.

Morphological parameters

To measure plant height 10 plants of sorghum were selected randomly from every sub-plot and measured their height from base to tip of the last leaf with measuring tape and calculated their average. Ten plants were selected randomly and removed their all green leaves. The leaves of every plant were counted and then average was calculated. For stem diameter, ten plants were selected randomly from each subplot and measured their stem diameter with Vernier calliper from top, middle and base of stem and was calculated the average stem diameter. Ten plants were selected randomly and removed their all green leaves and leaf area (cm^2) was determined by leaf area meter (Licor model 3100) and then leaf area per plant was measured. For fresh forage yield, all plants from each subplot were harvested and weighed with spring balance. Then fresh yield of sorghum was calculated in tonnes/ha. For dry matter yield (t ha^{-1}), a sample of plants was randomly selected from each subplot after harvesting the crop. These plants were chopped with a simple fodder chopper machine available at the University Farm. The chopped fodder was thoroughly mixed and took 500 g samples from each subplot. Sun drying was done for 3-4 days and then samples were dried in an oven at 70 ºC for three days to attain constant dry weight. After that the final dry weight was taken with the help of an electric balance.

Dry matter percentage

Dry matter % was determined by using the following formula:

$$\text{Dry matter (\%)} = \frac{\text{Dry matter yield}}{\text{Fresh weight}} \times 100$$

Dry matter yield (tons/ha) was calculated by dry matter % age and fresh fodder yield of sorghum.

Crude protein content (%)

Analysis of crude protein contents was done through a digestion process. First of all, dried sorghum sample was grinded in an electric grinder. Then a 1 g fine powder of sorghum sample was took in kjeldahl digestion flask and then 10 g of digestion mixture and 30 ml of concentrated H_2SO_4 were added in the digestion flask. After some time, the mixture was heated until a green liquid was obtained. After proper cooling, the green liquid was shifted into 250 ml volumetric flask and filled the flask with water up to the mark. Then 10 ml of this material, and 40% NaOH @ 15 ml was taken for each sample. Nitrogen was obtained in apparatus having 4% boric acid. Then methyl red and bromocresol green were added as indicators. Then the distilled material was titrated against N/10 H_2SO_4 until red color of solution was not obtained. Nitrogen was calculated from the amount of acid that was used in titration. Then the reading was multiplied with 6.25 to obtain crude protein percentage that was determined by recommended procedure of Estefan et al. (2013):

$$\text{Nitrogen percentage} = \frac{\text{A-B} \times 100 \times 100 \times 0.0014}{\text{Digested sample volume}}$$

Neutral detergent fiber contents (%)

To find out neutral detergent fiber (NDF) contents, 1 g fine powdered sample of sorghum was taken in the conical flask. After that 100 ml of NDF reagent and 0.50 g of sodium sulphate was added and flask was fitted with air condenser for temperature settlement. The flask was gradually heated for one hour. After that, the substances were permitted to cool and purified with suction pump. Remains were washed four times with hot water and then one time with acetone solution and then dried normally. Residues were shifted in a washed crucible and placed it in an oven for 1 hour at 105 °C. The crucible was placed in a desiccator for 10 minutes after that NDF was calculated by using the following formula described by Association of Official Analytical Chemist [AOAC], (2002):

$$\text{NDF (\%)} = \frac{\text{(Crucible wt + residues) – (Crucible wt)}}{\text{Wt of sample}} \times 100$$

Acid detergent fiber contents (%)

For acid detergent fiber (ADF) contents percentage, residues obtained from neutral detergent fiber was taken and mixed 100 ml of acid detergent solution in a conical flask of 500 ml of volume and the flask was attached with air condenser. The sample was heated till boiling point for 2-3 minutes and then reduced the temperature to reflux for 60 minutes. After boiling, the remaining residues were cleaned with suction pump and washed the contents for 2-3 times with hot distilled water and acetone. The remains were shifted in crucible and placed in an oven for about 24 hours at temperature of 105 °C, after drying, the condenser was used for proper cooling and then ADF contents were calculated (AOAC, 2002):

$$\text{ADF (\%)} = \frac{\text{(Crucible wt + ADF residues) – (Crucible wt)}}{\text{Wt of sample}} \times 100$$

Total ash content (%)

For total ash content (%), 5 g of powdered sorghum sample was taken in a china dish and placed the samples in a muffle furnace for 6-7 hours at 750 °C until grey ash contents were obtained. Then the sample was cooled in the desiccator, weighed (W1) and found the percentage ash contents (AOAC, 2002):

$$\text{Total ash content (\%)} = \frac{\text{W2 – W1}}{\text{Wt of sample}} \times 100$$

Calcium, zinc and iron contents (%)

For Ca, Zn and Fe contents (%), 1 g of powdered sorghum sample was taken in the Pyrex digestion tube of 100 ml, mixed the mixture of 10 ml of per chloric acid and nitric acid and then leaved the samples for 12 hours until all the reaction was over. After completion of digestion, the tube was placed in the desiccator for cooling and the temperature was increased for 1 hour up to 150 °C. U-shaped glass rod was attached under every funnel to remove vapours, and to raise the temperature gradually until all HNO_3 traces were disappeared. The glass rod was removed and the temperature was increased up to 235 °C. After appearance of white fumes, the digestion was continued till 30 minutes, the tubes were removed and cooled them in the desiccator and some water was added carefully. After that, Zn, Ca, and Fe were analysed by Atomic Spectrophotometer. The Ca was calculated by titration with EDTA. The calculations were completed according to a calibration curve (Estefan et al., 2013):

$$\text{Zn or Fe (ppm)} = \text{ppm Zn or Fe (from calibration curve)} \times \text{v/wt}$$

Where v = total volume of the plant digest (ml); wt = weight of dry plant (g)

Statistical analysis

Data of all parameters related to forage yield and quality was collected in the field and their statistical analysis was done by using Fishers' analysis of variance (ANOVA) and means of treatment were compared by using Tuckey's honestly significant difference (HSD) test at 5% level of probability (Steel et al., 1997).

Results and Discussion

Plant height (cm)

Sorghum is a fodder crop so height is the most important factor for crop production. Data revealed significant differences for plant height under different nutritional treatments (Table 2). Plant height varied from 202.55 cm to 263.38 cm. Maximum plant height (263.38 cm) was noted in combination of Ca at 3% + Zn at 2% + Fe at 1% while lowest plant height (202.55 cm) was observed under control treatment. Our results correlate with the findings of Haleema et al. (2018) who reported that interaction of Ca and Zn significantly increased plant height of tomato. Zinc and iron may increase photosynthetic rate and the ability of plants to obtain nutrients so improves the height of plants.

Stem diameter (cm)

Stem diameter is the main attribute of sorghum that indicates potential for green biomass and fodder yield. Data expressed significant differences for stem diameter under different bio-fortification treatments (Table 2). Stem diameter varied from 1.08 to 1.62 cm. Maximum stem diameter (1.62 cm) was observed when nutrients were applied in combination of Ca at 3% + Zn at 2% + Fe at 1% that was statistically similar with Zn at 2 % + Fe at 1 % and minimum stem diameter (1.08 cm) was noted in the control treatment (no spray) that was statistically at par with Ca at 3% and Fe at 1% treatments. These results are supported by the research findings of Adsul et al. (2016) who stated that $ZnSO_4$ + $FeSO_4$ significantly enhanced forage yield of sorghum (stem diameter, leaf area etc.). Yield improvement was mainly due to higher accessibility of Zn and Fe that made organic matter complexes from organic matter during fortification process and this process continued for a long duration and released beneficiary nutrients in slow rate in soil in such a way that nutrients remained available to plants throughout crop growth period (Kumar et al., 2019).

Number of leaves per plant

Leaf number per plant is the main forage yield calculating parameter. Plants with more leaves are considered better for forage, while leaf numbers are controlled genetically but influenced by some environmental factors (moisture, nutrients, temperature etc.). Number of leaves ranged from 11.6 to 17.8 per plant. Maximum leaves per plant (17.8) were counted, when all three nutrients (Ca at 3% + Zn at 2% + Fe at 1%) were applied in combination and least number of leaves (11.6) were noted in control treatment (no spray) (Table 2). These findings are similar to the results of Haleema et al. (2018) who described that bio-fortification (foliar spray) of Ca and Zn on tomato considerably increases number of leaves. These nutrients have prominent function in plant metabolic processes such as regulating catalase enzymes assembled with superoxide dismutase, as well as in photorespiration, glycolate metabolism and chlorophyll contents (Mousavi et al., 2012).

Leaf area per plant (cm^2)

Larger the green leaf area higher will be the forage yield. Animals also like the foliar parts of fodder. Data represented that leaf area of sorghum was significantly influenced by the application of forage nutrient treatments (Table 2). Highest leaf area per plant (7648.4 cm^2) was recorded when Ca at 3% + Fe at 1% + Zn at 2% were applied in combination, that was statistically at par with Zn 2% + Fe 1%, Ca 3% + Fe 1%, Ca 3% + Zn 2%, Fe 1%, and Ca 3%. Minimum leaf area (4315.4 cm^2) was recorded from the plot where no spray was done (control). Our findings are similar to the results of Durgude et al. (2019) who noted maximum leaf area of sorghum due to Fe and Zn application than all other treatments. It is also reported that an increase in Fe doses significantly increased the plant leaf area of Murcott (Orange hybrid) (Incesu et al., 2015). Increased area of leaf may be due to the fact that bio-fortification (foliar spray) of Fe and Zn increased the cell elongation, cell division, photosynthetic activity and metabolic rates (Adnan et al., 2020).

Fresh forage yield (t/ha)

Fresh yield is aggregate of all outcome characters like plant height, diameter of stem, plant density and leaf number. Data showed that fresh fodder yield of sorghum varied from 35.75 to 53.0 (t/ha). Combined application of Ca at 3% + Zn at 2% + Fe at 1% gave maximum (53.0 t/ha) fresh forage yield of sorghum and minimum (35.75 t/ha) yield was noted by control treatment (no spray) (Table 2). These research findings are similar with the results of Choudhary et al. (2017) who described that foliar application micronutrients (B + Fe + Zn) in combination increased economical, forage and biological yields. The increase in above parameter may be due to the fact that Fe is a constructive component of different enzymes (hematian, cytochroms, propyrin and ferrichrome) that favourably improves the nutritional environment of crop and final yield.

Dry matter yield (t/ha)

High dry matter yield is the indicator of good quality forage. Data expressed significant changes in dry matter yield under different bio-fortification treatments. DMY ranged from 7.3 to 16.12 t/ha. Maximum dry matter yield (16.12 t/ha) was noted when Ca at 3% + Zn at 2% + Fe at 1% was applied in combination whereas lowest dry matter yield was noted (7.3 t/ha) when plants were not sprayed by the nutrients (control treatment) (Table 2). These findings are also correlated with the research findings of Kobraee et al. (2011) who described that in soybean, collective application of Zn and Fe improved dry matter and yield of seed to great extent as compared to sole application. Bio-fortification of Fe, Zn and Boron improved the yield and increase in yield might be due to their crucial role in photosynthesis, respiration, biochemical and physiological activities (Salih, 2013).

Table 2 Influence of bio-fortification of Ca, Zn and Fe on yield and yield components of forage sorghum

Treatment	Plant height (cm)	Stem diameter (cm)	Number of leaves/plant	Leaf area/plant (cm^2)	Fresh forage yield (t/ha)	Dry matter yield (t/ha)
Control	202.55[c]	1.09[d]	11.59[c]	4315.4[c]	35.75[d]	7.3[d]
Ca 3%	221.50[bc]	1.24[cd]	15.33[ab]	6051.5[b]	41.4[c]	11.3[c]
Zn 2%	247.35[ab]	1.38[bc]	15.83[ab]	6514.7[ab]	44.5[b]	11.4[c]
Fe 1%	224.90[bc]	1.26[cd]	14.68[b]	6756.0[ab]	39.8[c]	11.2[c]
Ca 3% + Zn 2%	239.93[ab]	1.32[bc]	15.05[b]	6967.5[ab]	45.0[b]	11.4[c]
Ca 3% + Fe 1%	228.90[bc]	1.38[bc]	15.70[ab]	6677.7[ab]	41.3[c]	10.3[c]
Zn 2% + Fe 1%	252.08[ab]	1.50[ab]	15.45[ab]	7093.8[ab]	46.3[b]	12.91[b]
Ca 3% + Zn 2% + Fe 1%	263.38[a]	1.62[a]	17.80[a]	7648.4[a]	53.0[a]	16.12[a]

Means showing different letters differed significantly ($p \leq 0.05$)

Crude protein contents (%)

Data regarding crude protein contents of forage sorghum showed significant differences by the foliar application of different nutrients. Crude protein % ranged from 10.23 to 18.27% (Fig. 1). Maximum crude protein (18.27%) was recorded when Ca 3% + Zn 2% + Fe 1% were applied in combination that was statistically similar with Zn 2% + Fe 1% and minimum crude protein (10.23%) was obtained by control treatment. These findings are parallel with the results of Chand et al. (2017) who described that soil application of $ZnSO_4$ at 25 kg/ha + foliar spray of $ZnSO_4$ at 2% gave maximum crude protein contents. Similarly, the protein contents were increased by the application of Zn (Tahir et al., 2016). El-Aal and Eid (2018) also investigated that application of minerals (N, P, K, Fe, Ca and Mg) highly increased the total carbohydrates and crude protein% in soybean. Improved protein contents might be due to better uptake of nutrients which is an integral part of protein synthesis (Balai et al., 2011).

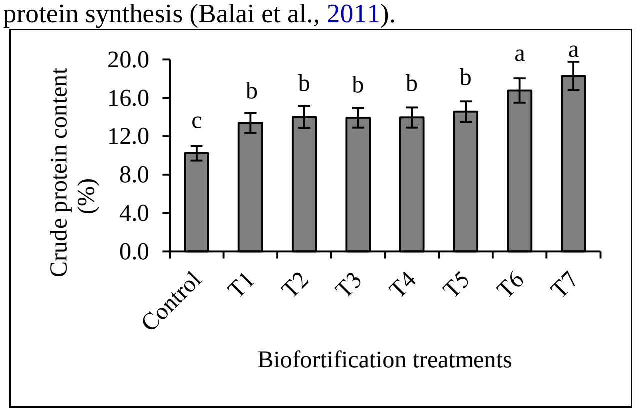

Fig. 1 Influence of bio-fortification of Ca, Zn and Fe on crude protein contents (%) of forage sorghum

Ash content (%)

Data regarding total ash contents showed that ash contents of sorghum were significantly influenced by the foliar nutrients application (Fig. 2). Ash content noted between 9.6 to 21.3%. Maximum ash contents were recorded when combined application of Ca 3% + Zn 2% + Fe 1% was done which was statistically at par with all other nutritional treatments. The control (no spray) gave minimum ash contents. These results are confirmed by Soleymani and Shahrajabian (2012) who stated that bio-fortification of manganese, Zn and Fe increased ash contents of sorghum. Micronutrients, particularly Fe behave as metal components of numerous enzymes and are also related with photosynthesis, saccharide metabolism and protein synthesis (Rout & Sahoo, 2015).

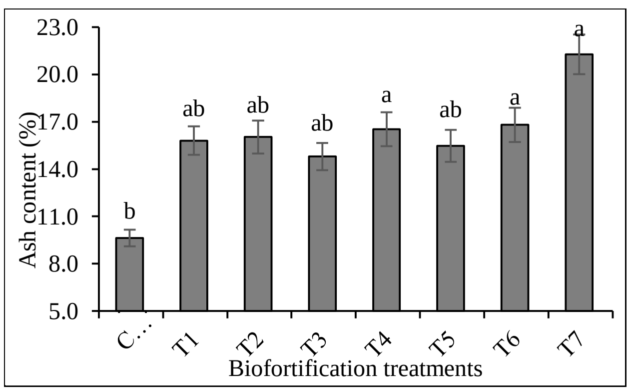

Fig. 2 Influence of bio-fortification of Ca, Zn and Fe on ash contents (%) of forage sorghum

Neutral detergent fiber (NDF) contents (%)

Neutral detergent fiber contents are the plant's structural components, specifically the cell wall. Good quality forages generally have low NDF contents. Data indicates that NDF contents were between 67.80 to 78.67% (Fig. 3). Maximum NDF contents (78.67%) were obtained from control treatment and minimum NDF contents were obtained (67.80%) by combined application of Ca 3% + Zn 2% + Fe 1 % which was statistically at par with Zn 2% + Fe 1% (Fig. 3). These results are confirmed by Chand et al. (2017) who observed reduction in NDF and ADF contents of Baby Corn *(Zea mays* L.) due to application of Zn along with recommended dose of fertilizer.

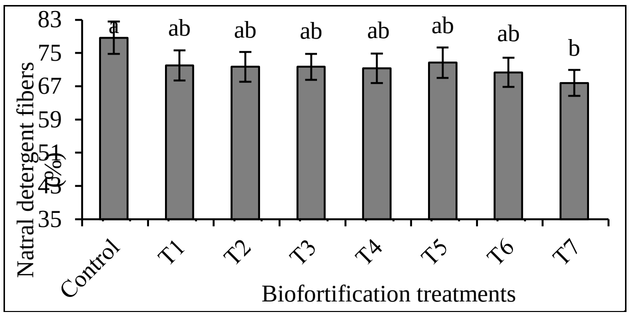

Fig. 3 Influence of bio-fortification of Ca, Zn and Fe on NDF contents (%) of forage sorghum

Acid detergent fiber (ADF) contents (%)

Data related to ADF contents of sorghum showed that ADF contents ranged from 31.67 to 41% (Fig. 4). Maximum ADF contents (41.00%) were observed in control (no spray) and lowest ADF contents (31.67%) were recorded when Ca 3% + Zn 2% + Fe 1% were applied in combination. These results are similar with the findings of Sheta et al. (2010) who reported that higher levels of fertilizers significantly reduced ADF and neutral detergent fiber contents. This may be due to the reason that Zn reduces the fibre and soluble carbohydrate content in fodders thereby increases digestibility (Tsonev & Cebola Lidon, 2012).

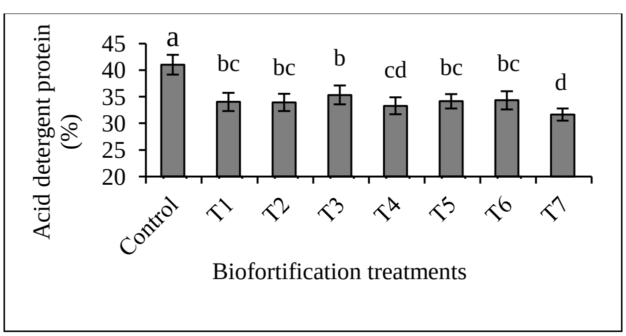

Fig. 4 Influence of bio-fortification of Ca, Zn and Fe on ADF contents (%) of forage sorghum

Sorghum calcium (Ca) contents (mg/kg)

Mean comparison data for Ca contents affected due to the bio-fortification treatments (Fig. 5). Calcium contents ranged from 0.56 to 1.8 mg/kg dry weight of sorghum. Maximum Ca contents (1.13) were obtained when Ca at 3% was applied and minimum Ca contents (0.96) were recorded when Zn at 2% + Fe at 1% was applied on plants that were statistically at par with control (Table 3). Similar results were presented by Dayod et al. (2010) who observed that application of Ca increases the Ca contents in plants.

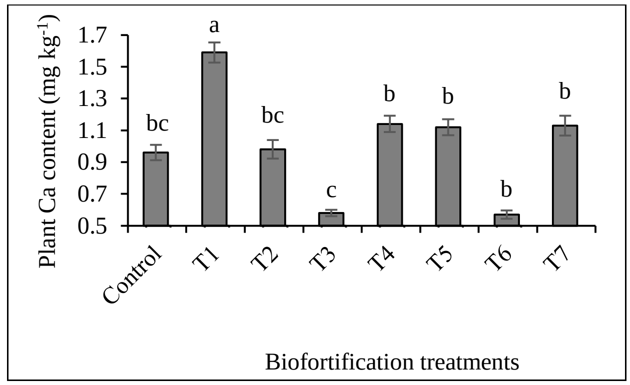

Fig. 5 Influence of bio-fortification of Ca, Zn and Fe on sorghum Ca contents (%) of forage sorghum

Sorghum zinc (Zn) contents (mg/kg)

Data regarding Zn contents of forage sorghum affected by foliar nutrients application expressed that Zn contents ranged 63.50 to 142.91 mg/kg dry weights of sorghum (Fig. 6). Maximum Zn contents (142.91 mg/kg) were recorded by combined application of Ca at 3% + Zn at 2% + Fe at 1% that is statistically similar with application of Zn at 2% + Fe at 1% and Zn at 2% and minimum Zn contents (63.50 mg/kg) were recorded when 3% Ca was applied. These findings correlate with the research findings of Qadir et al. (2017) who noted that Zn and Fe concentration was greatly improved by foliar fertilization of Zn and Fe. So, there is the direct and positive correlation among Zn and Fe.

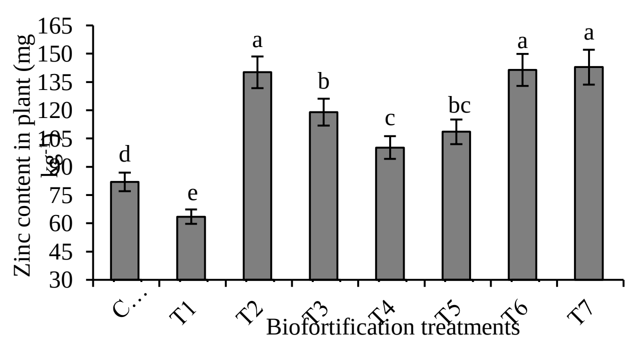

Fig. 6 Influence of bio-fortification of Ca, Zn and Fe on Zn contents (%) of forage sorghum

Sorghum iron (Fe) contents (mg/kg)

Data showed that Fe contents varied from 16.43 to 47.29 mg/kg dry weight of sorghum (Fig. 7). Maximum Fe contents (47.29 mg/kg) were noted when Zn at 2% + Fe at 1% were applied that was statistically at par with Fe at 1% and Ca at 3% + Zn at 2% + Fe at 1 % whereas minimum Fe contents (16.43 mg/kg) were obtained by the application of Ca at 3% that is statistically at a par with control treatment. These findings are confirmed by the finding of Xia et al. (2019) who described that application of Zn fertilizer had positive impact on Fe contents of plant.

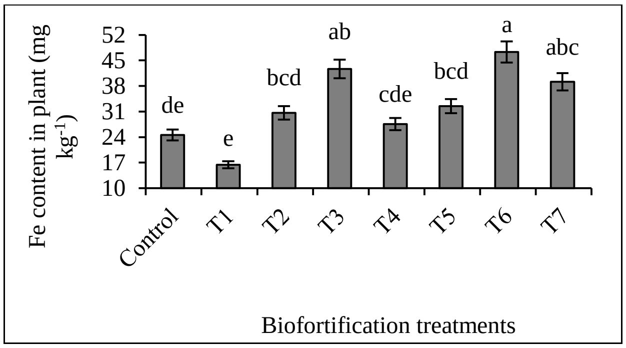

Fig. 7 Influence of bio-fortification of Ca, Zn and Fe on sorghum Fe contents (%) of forage sorghum

Conclusion

Foliar application of Ca, Zn and Fe is a good strategy to enhance the yield and quality of sorghum fodder and combined foliar application of Ca + Zn + Fe at the rate of 3%, 2% and 1% respectively at 4 leaf stage is suggested for improving yield and quality of forage sorghum.

Author Contribution Statement: Muhammad Asif planned and supervised the experiment, Basharat Abbas involved in planning and execution of experiment, Ahsan Aziz participated in the discussion of the study, Muhammad Adnan participated in the statistical analysis, methodology and discussion of the study, Ali Raza participated in write up of manuscript, Muhammad Ehsan Safdar involved in drafting the tables, Amjed Ali involved in drafting the figures and Muhammad Shakeel Hanif reviewed the write up of manuscript.

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Conflict of Interest: The authors declare no conflict of interest.

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