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Effect of Sulfur and Molybdenum on Quality of *Kharif* Forage Cowpea (*Vigna unguiculata* (L.) Walp.)

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Abstract The field experiment was conducted to find out the effect of sulfur and molybdenum on quality of *kharif* forage cowpea (*Vigna unguiculata* (L.) Walp.) during *kharif* season of 2013. The experiment was laid out in factorial randomized block design, comprising 16 treatments combinations of four levels each of S (0, 20, 40 and 60 kg ha^{-1}) and Mo (0, 0.5, 1.0 and 1.5 kg ha^{-1}), which were replicated 4 times. The observations of quality parameters included crude protein%, NDF%, ADF%, hemicelluloses and IVDMD% were recorded after harvesting of crop. The application of sulfur and molybdenum at varying levels did not significantly effect on crude protein%, NDF%, ADF% and hemicelluloses contents of forage cowpea except ADF% due to sulfur levels, while IVDMD% was significantly affected due to both sulfur and molybdenum application. With the increasing levels of sulfur there was decreased in ADF%, while IVDMD% was increased as the application rate was increased. The decreased in ADF% under S_{60} was to the extent of 8.8%, while increase in IVDMD% was 4.4% over control (S_0).

Keywords Cowpea, Sulfur, Molybdenum, Crude protein, NDF.

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Introduction

In India, cowpea is grown in about 0.25 million hectares for different purpose. In Gujarat, at present fodder availability is about 20 million tones as against the requirement of 49.2 million tonnes [1]. Leguminous fodder seems to be the cheapest source of protein to supplement the grasses and straw available in the state. Green forage not only helps for easy digestion, but also supplies abundant quantity of the Vitamin-'A' and important minerals like Ca and Fe for the animals. The cowpea (*Vigna unguiculata* L. Walp.) commonly known as 'Lobia' used as a pulse, fodder, green pod vegetable and green manure crop is rich in protein and containing many other nutrients and hence, it is known as vegetable meat. On dry weight basis, cowpea grains contain 23.4% protein, 1.8% fat and 60.3% carbohydrates. It is used for both human consumption and as a concentrate feed for cattle. The

Table 1. Detail of treatments on forage cowpea.

Treatments	Sulfur (S) (kg ha^{-1})	Molybdenum (Mo) (kg ha^{-1})	Treatment combination
T_1 (control)	0	0.0	$S_0Mo_{0.0}$
T_2	0	0.5	$S_0Mo_{0.5}$
T_3	0	1.0	$S_0Mo_{1.0}$
T_4	0	1.5	$S_0Mo_{1.5}$
T_5	20	0.0	$S_{20}Mo_{0.0}$
T_6	20	0.5	$S_{20}Mo_{0.5}$
T_7	20	1.0	$S_{20}Mo_{1.0}$
T_8	20	1.5	$S_{20}Mo_{1.5}$
T_9	40	0.0	$S_{40}Mo_{0.0}$
T_{10}	40	0.5	$S_{40}Mo_{0.5}$
T_{11}	40	1.0	$S_{40}Mo_{1.0}$
T_{12}	40	1.5	$S_{40}Mo_{1.5}$
T_{13}	60	0.0	$S_{60}Mo_{0.0}$
T_{14}	60	0.5	$S_{60}Mo_{0.5}$
T_{15}	60	1.0	$S_{60}Mo_{1.0}$
T_{16}	60	1.5	$S_{60}Mo_{1.5}$

Table 2. Composition of McDougall buffer used for *in vitro* studies.

Sl. No.	Particular	Quantity
Solution A (Micro minerals) for 100 ml		
1.	Calcium chloride ($CaCl_2.2H_2O$)	13.2 g
2.	Manganese chloride ($MnCl_2.4H_2O$)	10.0 g
3.	Cobalt chloride ($CoCl_2.6H_2O$)	1.0 g
4.	Iron chloride ($FeCl_2.6H_2O$)	8.0 g
5.	Distilled water (final volume)	To make 100 ml)
Solution B (Buffer solution)		
1.	Sodium hydrogen carbonate ($NaHCO_3$)	35.0 g
2.	Ammonium hydrogen carbonate ((NH_4) HCO_3)	4.0 g
3.	Distilled water (final volume)	To make 1000 ml
Solution C (Macro minerals)		
1.	Disodium hydrogen phosphate (Na_2HPO_4)	5.7 g
2.	Potassium dihydrogen phosthate (KH_2PO_4)	6.2 g
3.	Magnesium sulfate ($MgSO_4.7H_2O$)	0.6 g
4.	Distilled water (final volume)	To make 1000 ml
Resazurin solution		
1.	Resazurin	100 mg
2.	Distilled water (final volume)	To make 100 ml
Reducing solution		
1.	Sodium hydroxide (1N NaOH)	4 ml
2.	Sodium sulfide ($Na_2S.9H_2O$)	625 mg
3.	Distilled water (final volume)	To make 100 ml

crop forms excellent forage. Cowpea is often grown as a green manure for soil improvement. The crop gives such a heavy vegetative growth and covers the ground so well that it checks the soil erosion in problem areas and can later be ploughed in as green manure. It has considerable promise as an alternative pulse crop in dry land farming [2]. For the purpose of forage it is grown as mixed crop with sorghum and pearl millet as well as a sole crop. Now a days, traditional boundaries between primary and secondary nutrients are becoming narrower. Crop requires as much sulfur as they require phosphorus hence, it can be considered as the fourth major nutrient and interacts with both macro and micro-nutrients. The wide sperad sulfur deficiency is prevailing in cultivable land which ranged from 11 to 64% with an overall mean of 36.5% in soils of different states of India. About 37% soils of Gujarat are sulfur deficient. Thus, sulfur deficiencies can only be corrected by the application of sulfur fertilizer [3]. Sulfur is also a part enzymes like nitrogenase. It is a constituent of free amino acids such as methionine and cysteine [4] and plays a vital role in protein synthesis. Sulfur is essential for chlorophyll formation, synthesis of protein, vitamins, thiourea, plant hormones, thiamin, biotin, glutathione and S-containing essential amino acids viz., methionine, cystine and cysteine. Sulfur increase crop yields and improve quality of produce, both of which are important for determining the market price. After phosphorus, sulfur nutrition has been found to be a major limiting factor in green gram production. Molybdenum is a trace element found in the soil and is required for growth of most of the biological organisms including plants and animals. Legumes generally have higher Mo requirements than grasses. "Molybdenum" had beneficial effect on legumes and little effect on net assimilation rate in green gram, increased it slightly in black gram and cowpea and increased it 3 fold in cluster beans. Dry matter yield and nitrogen-fixing enzyme nitrogenase found in bacteroids of legume cowpea/pot increased due to Mo application up to 5 mg Mo/kg of soil [5]. When plants are grown under molybdenum deficiency, a number of varied

Table 3. Composition of media for *in vitro* studies.

Particulars	Quantity of different solutions and SRL			
Solution A (Micro) (ml)	0.10	0.12	0.14	0.15
Solution B (Buffer) (ml)	190.23	237.78	285.34	309.12
Solution C (Macro) (ml)	190.23	237.78	285.34	309.12
Resazurin solution (ml)	0.95	1.19	1.43	1.55
Reducing solution (ml)	38.05	47.56	57.07	61.82
Distilled water (ml)	380.45	475.57	570.68	618.24
Total media (ml)	800	1000	1200	1300
Rumen liquor (ml)	400	500	600	650
Total mixture (ml)	1200	1500	1800	1950

Table 4. Effect of sulfur and molybdenum on crude protein content and quality attributing character of forage cowpea.

Treatments	Crude protein (%)	NDF (%)	ADF (%)	Hemice-lluloses (%)	IVDMD (%)
Sulfur levels (kg ha^{-1})					
S_0 (control)	12.25	61.56	48.75	12.44	68.01
S_{20}	12.51	59.38	46.94	12.81	69.95
S_{40}	12.55	58.94	45.63	13.31	70.88
S_{60}	12.57	58.81	44.81	14.00	70.99
SEm±	0.12	1.01	1.02	0.42	0.38
CD (p=0.05)	NS	NS	2.89	NS	1.07
Molybdenum levels (kg ha^{-1})					
Mo_0	12.38	59.88	47.00	12.88	68.57
$Mo_{0.5}$	12.51	60.38	47.44	12.94	70.77
$Mo_{1.0}$	12.59	58.81	45.25	13.56	70.80
$Mo_{1.5}$	12.40	59.63	46.44	13.19	69.69
SEm±	0.12	1.01	1.02	0.42	0.38
CD (p=0.05)	NS	NS	NS	NS	1.07
Interaction (S×Mo)					
CD (p=0.05)	NS	NS	NS	NS	Sign.
CV %	3.77	6.75	8.73	12.78	2.15

phenotypes develop that hinder plant growth. Most of these phenotypes are associated with reduced activity of molybdoenzymes. These enzymes include the primary nitrogen assimilation enzymes such as nitrate reductase (NR) nodules. The research work on this aspect particularly for fodder production of summer cowpea is more useful. When S and Mo applied together may act in synergistic manner which may have a direct influence on the yield and biochemical composition of grain. These will be very helpful to the more production of fodder with respect to the quality as well as the quantity of forage cowpea.

Materials and Methods

A field experiment was conducted during the *kharif* season of the year 2013 in Plot No. A-15-2 at Main Forage Research Station, Anand Agricultural University, Anand, Gujarat. The climate of Anand region is semi-arid and sub-tropical with hot summer and mild cold winter. Experimental field had an even topography with a gentle slope and good drainage system. The soil is representative of the region (Alluvial nature) and is locally known as "*Goradu*" soil. The texture of the soil is loamy sand. The soil is very deep and fairly moisture retentive. The soil of experimental plot was low in available nitrogen, medium in available phosphorus and available potash.

The experimental field was laid out in factorial randomized block design (FRBD) with four replication and follows the recommendations agronomic intercultural practices during the growing period of forage cowpea variety Gujarat Forage Cowpea-1 (GFC-1) (Table 1). The seed were treated with imidachloprid 17.80% SL before the sowing.

Fertilizer application

The crop was fertilized as per the respective treatments i.e. 0, 20, 40 and 60 kg Sulfur ha^{-1} in the form of Gypsum and 0.0, 0.5, 1.0 and 1.5 kg Molybdenum ha^{-1} in the form of Ammonium Molybdate. The full dose of nitrogen @ 20 kg N ha^{-1} in the form of urea and Phosphorus @ 40 kg P_2O_5 ha^{-1} in the form of Diammonium Phosphate (DAP) was applied common as basal dose at the time of land preparation.

The quality parameters analyzed after harvesting of crop which included crude protien content (%), acid detergent fiber content (ADF%),neutral detergent fiber content (NDF%), hemicellulose content and *in-vitro* dry matter digestibility. The crude protein content (%) was estimated from the powder of representative oven dried samples using Near-Infrared spectroscopy method (NIR analyzer). Thoroughly ground and mixed dry sample of each treatment was

Table 5. Interaction effect of S × Mo on *in vitro* dry matter digestibility content (%) at harvest.

Mo / S	*In vitro* dry matter digestibility content (%) Mo$_0$ (control)	Mo$_{0.5}$	Mo$_{1.0}$	Mo$_{1.5}$
S$_0$ (control)	67.50	70.15	68.51	65.87
S$_{20}$	67.11	72.45	69.94	70.29
S$_{40}$	70.37	70.41	72.72	70.02
S$_{60}$	69.28	70.05	72.05	72.59
SEm ±		0.75		
CD (p=0.05)		2.14		

taken for estimation of acid detergent fiber (ADF%) content and neutral detergent fiber (NDF%) content [6]. The hemicellulose content (%) was worked out by employing the following formula:

Hemicellulose content (%) = Neutral detergent fiber (NDF) – Acid detergent fiber (ADF)

In vitro Dry Matter Digestibility (IVDMD%)

Experimental Animals

Two non-producing cows of same age and breed were selected as donor of rumen inoculums for *in vitro* studies and were kept in a well ventilated, hygienic and protected shed with individual feeding mangers. The dietary requirements of the donor animals were met as per standards mentioned by ICAR [7].

Collection of Rumen Liquor

Rumen liquor was collected at 2 h post feeding through a stomach tube against negative pressure created by a suction pump. The collected rumen liquor was strained through four layered muslin cloth and was referred as Strained Rumen Liquor (SRL). The SRL was brought to the laboratory in a pre-warmed (39 ± 1°C) thermos flask. Carbon dioxide gas was passed through the SRL for one minute and was maintained at 39 ± 1°C temperature for further analysis. The artificial saliva (McDougall buffer) for *in vitro* studies was prepared fresh. The composition of atrificial saliva is given in Tables 2 and 3 for *in vitro* studies.

The oven dried samples of forage cowpea variety GFC-1 grown on soil supplemented with varying levels of Sulfur and Molybdenum will be were subjected to determination of *in vitro* dry matter digestibility. Finely ground 500 mg of fodder samples were taken into 100 ml calibrated glass syringe in 4 replication [8]. For *in vitro* studies, 10 ml of SRL along with 40 ml of fresh McDoughall buffer was added to the syringes containing substrate. The syringes containing substrate along with rumen liquor and buffer were incubated at 39 ± 1°C for 48 h in a shaking water bath. After 48 h of incubation, the content of each syringe was filtered through pre-weighed Gooch crucible dried and weighed. Simultaneously, the blank was also incubated without sample.

Determination of in vitro Digestibility

The *in vitro* dry matter digestibility (IVDMD) was calculated by the following equation:

$$\text{\% Dry matter digestibility} = \frac{\text{Initial DM of fodder taken for incubation} - \text{DM in residue} \times 100}{\text{Initial DM of fodder taken for incubation}}$$

Results and Discussion

The data on Crude protein content, NDF, ADF, Hemicelluloses and IVDMD (%) of forage cowpea as influenced by different sulfur and molybdenum levels are summarized in Table 3.

Effect of sulfur levels

The application of sulfur at varying levels did not significantly affect crude protein content, neutral detergent fibre content (NDF%) and hemicelluloses content (%) (Table 4) but data on acid detergent fibre content (ADF%) and IVDMD% showed significant difference due to sulfur levels. An application of sulfur @ 0 kg ha^{-1} (S$_0$) recorded significantly higher acid detergent fibre content (48.75%) than higher levels of S (S$_{40}$ and S$_{60}$), however it was at par with S$_{20}$ (46.94%).

The lowest acid detergent fibre content (44.81%) was recorded under S_{40}. The decrease in NDF% was to the extent of 58.81% (S_{60}) over S_0. The sulfur @ 60 kg ha^{-1} (S_{60}) gave significantly higher *in vitro* dry matter digestibility content (70.99%) than S_0 (68.01%), but it was at par with S_{40} (70.88%) and S_{20} (69.95%) levels. The increase in *in vitro* dry matter digestibility content (IVDMD%) under S_{60}, S_{40} and S_{20} was to the extent of 4.38, 4.22 and 2.85%, respectively over S_0 (control). Sulfur plays a dynamic roles in plant growth and metabolism in terms of their action on catalytic or electrochemical function of biomolecules in the cells [9].

Sulfur containing amino acids cysteine, cystine and methionine helps in the development of complete proteins and which is most important for the pulses crops. In the present investigation, sulfur addition at varying levels increased crude protein content at each increasing levels, the difference were not significant [10]. An application of 60 kg S ha^{-1} synthesized the lowest NDF content. The reduction in NDF content was observed with increase in the level of sulfur which might be due to increase in succulence i.e. leaf: stem ratio of plant by reducing formation of polysaccharides viz., cellulose, hemi-cellulose and lignin, which generally account for NDF content in the plant [11].

Effect of molybdenum levels

The application of molybdenum at varying levels did not affect on crude protein, neutral detergent fiber (NDF%), acid detergent fiber (ADF%) and hemicelluloses content (%) significantly, while *in vitro* dry matter digestibility content (IVDMD%) were significantly improved due to Mo application. The application of 1 kg ha^{-1} molybdenum (Mo_3) gave significantly higher percent of *in vitro* dry matter digestibility content (70.80%) than control (Mo_0), rest of the levels were at par. The increase in *in vitro* dry matter digestibility content (IVDMD%) under $Mo_{1.0}$ and $Mo_{0.5}$ was to the extent of 3.25 and 3.21%, respectively over no molybdenum (Mo_0).

Interaction effect

The *in vitro* dry matter digestibility content (IVDMD%) was significantly altered by interaction effect of sulfur and molybdenum (Table 5). The results revealed that among different combinations, $S_{40}Mo_{1.0}$ combination registered maximum value (72.72%) of IVDMD, which was significantly higher than the rest of the combinations barring $S_{60}Mo_{1.5}$ and $S_{60}Mo_{1.0}$ combinations. The lowest *in vitro* dry matter digestibilty content (65.87%) was recorded under the combination of $S_0Mo_{1.5}$.

Conclusion

The lower acid detergent fiber (44.81 and 45.25%) and neutral detergent fiber content (58.81 and 58.81%) was recorded under the application of sulfur (S_{60}) and molybdenum ($Mo_{1.5}$) level, respectively as compared to the other levels. Moreover, the higher crude proteins (12.57 and 12.59%), hemicellulose (14.00 and 13.56%) and *in vitro* dry matter digestibility (70.99 and 70.80%) was observed under the level of sulfur @ 60 kg ha^{-1} and molybdenum @ 1.0 kg ha^{-1}, respectively. The results revealed that among different combinations, $S_{40}Mo_{1.0}$ combination registered maximum value (72.72%) of IVDMD, which was significantly more than the rest of the combinations barring $S_{60}Mo_{1.5}$ and $S_{60}Mo_{1.0}$ combinations. The lowest *in vitro* dry matter digestibility content (65.87%) was recorded under the combination of $S_0Mo_{1.5}$. Therefore, the quality of forage cowpea which are use as animal feeding can be improve through the soil application of sulfur and molybdenum.

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