INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume-1, Issue-3 Sept-Nov-2011 www.ijpaes.com *ISSN 2231-4490*

NITRATE ACCUMULATION IN SORGHUM (*SORGHUM BICOLOR*) AS INFLUENCED BY SULPHUR AND PHOSPHORUS APPLICATION UNDER INCREASING NITROGEN LEVELS

SUCHHANDA MONDAL[a]* and B. K. NAD[b]

[a] Assistant Professor, Institute of Agriculture, Visva-Bharati, Sriniketan-731236, West Bengal, India

[b] Principal Scientist (Retd.) Indian Agricultural Research Institute, New Delhi 110012. India

* Corresponding author, e-mail: suchhandamondal@rediffmail.com

ABSTRACT: A green house experiment was conducted to study the effect of phosphorus and sulphur under increasing levels of nitrogen on NO_3-N accumulation by sorghum. Three levels of nitrogen (60, 120 and 240 kg N ha^{-1}), two levels of phosphorus (0 and 90 kg P_2O_5 ha^{-1}) and two levels of sulphur (0 and 45 Kg ha^{-1}) comprised the treatment combinations. At 35 and 70 DAS, plants were harvested and analyzed for NO_3-N, total N, P, K, S, Ca and Mg contents. Total nitrogen uptake increased with increasing nitrogen application and application of sulphur and phosphorus. NO_3-N content was also increased with increasing rate of nitrogen application but decreased with application of sulphur and phosphorus, and with age. Phosphorus and sulphur application increased P, S, K, Ca and Mg uptake. Nitrogen application at increasing rate increased the per cent sulphur derived from fertilizer and per cent fertilizer sulphur utilization, however phosphorus caused a reduction in both the values.

Key words: Nitrate accumulation, Phosphorus, Sorghum, Sulphur.

INTRODUCTION

Nitrogen is the element that most limits crop growth. It is particularly important in forage crops to attain a luxurious growth with high palatability. However, non-judicious application of nitrogenous fertilizer can cause free nitrate accumulation in fodder crops and animals feed on them may suffer from toxicity. Rumen microbes normally convert NO_3^- to NO_2^- and if NO_3^- supply exceeds the capacity of microbes to convert NO_2^- to ammonia, NO_2^- concentration in blood rises. Nitrite ties up haemoglobin and thereafter the oxygen carrying capacity of blood is reduced which may result in suffocation (Kemp *et al.,* 1977). High nitrate intake can also affect iron containing enzymes other than haemoglobin (Johnson *et al.,* 1983). Although nitrogen supply is the most important factor governing nitrate accumulation in vegetative parts of plants, it can, at least to some extent, be controlled through balanced fertilization with other nutrients, particularly phosphorus and sulphur which are considered as helpful in reducing nitrate to ammonia for proper assimilation. Time is also an important factor governing nitrate accumulation. Free nitrates accumulated by young plants gradually decrease due to its subsequent reduction and assimilation with maturity of the plant. Hence, to minimize the risk of nitrate poisoning in ruminants, balanced fertilization along with increasing nitrogen levels and maintaining a proper harvesting time may be beneficial without compromising with the yield. The present study was, therefore, undertaken to study the effects of increasing rates of nitrogen application along with balanced fertilization with phosphorus and sulphur on nitrate accumulation in two growth stages of sorghum.

MATERIALS AND METHODS

A pot culture experiment was conducted at Indian Agricultural Research Institute, New Delhi, having Sorghum ("PCH 9", fodder sorghum hybrid) as the test crop. The soil used was sandy loam in texture with slightly alkaline reaction. It contained 0.41% organic carbon, 228.6 kg ha^{-1} available nitrogen, 23.32 kg ha^{-1} available phosphorus, 229 kg ha^{-1} available potassium and 32.67 kg ha^{-1} available sulphur. The treatments comprised three levels of nitrogen *viz*.,60, 120 and 240 kg ha^{-1} (i.e., 26.78, 53.57 and 80.36 mg N kg^{-1} soil, respectively), two levels of phosphorus *viz*, 0 and 90 kg P_2O_5 ha^{-1} (i.e., 0 and 40.18 mg P_2O_5 kg^{-1} soil) and two levels of sulphur *viz,* 0 and 45 kg ha^{-1} (i.e., 0 and 20.9 mg S kg^{-1} soil). A basal dose of potassium @ 60 kg ha^{-1} (26.78 mg kg^{-1} soil) was applied in all the pots. The sources of nitrogen, phosphorus, sulphur and potassium were urea, mono-ammonium phosphate (MAP), ammonium sulphate (AS) and muriate of potash (MOP), respectively. The source of sulphur was labeled with ^{35}S with a tagging rate of 0.4 m Ci g^{-1} S).The amounts of nitrogen supplied through monoammonium phosphate and ammonium sulphate were balanced to supply required nitrogen dose through urea. Plants were harvested at two stages, i.e., at 35 and 70 days after sowing. Plant samples were analyzed for NO_3-N, total N, P, K, S, Ca and Mg contents. NO_3-N was extracted by the procedure of Grover *et al.* (1978) and determined colorimetrically following the same procedure as was prescribed by Jackson (1973) for soil. ^{35}S activity in plant digest was counted in liquid scintillation counter. Per cent sulphur derived from fertilizer (% Sdff) and per cent utilization of fertilize sulphur were calculated as

$$\%Sdff = \frac{\text{Specific activity of sample at zero hour}}{\text{Specific activity of standard at zero hour}} \times 100$$

$$\text{Per cent utilization of fertilizer sulphur} = \frac{\%\ \text{Sdff} \times \text{total S uptake}}{\text{Rate of fertilizer S application}}$$

RESULTS AND DISCUSSION

NO_3-N content

There was more than three-fold increase in NO_3-N content due to increasing nitrogen application from N_{60} to N_{240}. It varied between 260 and 888 mg kg^{-1} at 35 DAS (Table 1). The highest value was obtained in pots receiving the highest level of nitrogen with no phosphorus and sulphur. This value was reduced to 611 mg kg^{-1} for the same nitrogen level when phosphorus and sulphur were also applied. Yoon and Choi (1999) showed that accumulation of NO_3-N in sorghum occurred at 200 kg N ha^{-1} and exceeded the safe level for ruminants in Korea at 400 kg N ha^{-1}. Both phosphorus and sulphur were able to reduce NO_3-N content in plant at both the stages. Application of sulphur reduced NO_3-N content from 8189 to 643 mg kg^{-1} at the highest level of nitrogen at 35 DAS. At the second stage (70 DAS), the plant NO_3-N content reduced greatly ranging between 260 to 487 mg kg^{-1}. Application of nitrogen alone maintained a higher level of NO_3-N content in plant for all the three levels. The reduction in NO_3-N conc. with time is due to the fact that at initial stages roots are more active in nutrient uptake and often accumulates nitrate in excess amount. With advancement in growth, plant is able to convert more nitrates to amino acids (Crawford *et al*., 1965).

Nitrogen content and uptake

Total nitrogen content of sorghum varied due to nitrogen and sulphur application at 35 DAS. The maximum value of 20.91 mg N g^{-1} was recorded in pots receiving the highest dose of nitrogen along with phosphorus and sulphur (Table 1). At 70 DAS application of nitrogen also caused an increase in nitrogen content up to N_{120}. Further increase in nitrogen had no significant effect. At this stage, phosphorus application caused a reduction in nitrogen content whereas, application of sulphur had no significant effect on it. The overall values were much lower compared to the first stage.

Like nitrogen content, nitrogen uptake was also increased with increase in nitrogen levels up to the highest dose (Table 1). Individual application of sulphur and phosphorus caused increase in nitrogen uptake. Total nitrogen uptake was increased by more than two-fold during the second sampling (70 DAS).

Table 1.NO_3-N content (mg kg^{-1}), total-N content (mg g^{-1}), total N uptake (mg pot^{-1}) and P uptake (mg pot^{-1}) of sorghum as influenced by the applications of N, P and S

Treatments	NO_3-N content		Total N content		Total N uptake		P uptake	
	35 DAS	70 DAS	35 DAS	70 DAS	35 DAS	70 DAS	35 DAS	70 DAS
N_{60}	281	265	15.54	6.77	92.5	250.9	13.18	64.24
N_{120}	565	343	16.75	7.26	111.6	287.4	16.10	74.86
N_{240}	731	408	20.05	7.87	159.7	401.6	13.48	70.76
CD	51	52	1.68	0.63	13.5	41.6	1.57	6.51
P_0	554	363	16.86	7.72	112.5	299.2	12.69	58.69
P_{90}	526	314	17.63	6.87	130.1	335.9	15.82	81.20
CD	42	46	NS	0.51	11.0	33.9	1.28	5.31
S_0	575	371	16.39	7.46	113.1	328.9	13.17	67.12
S_{45}	477	306	18.10	7.13	129.5	306.3	15.33	72.77
CD	42	46	1.37	NS	11.0	NS	1.28	5.31
$N_{60}P_0$	296	274	14.89	7.43	84.7	258.5	12.07	58.54
$N_{120}P_0$	586	371	15.74	7.54	104.4	282.1	14.64	61.71
$N_{240}P_0$	781	445	19.91	8.19	148.3	357.8	11.36	55.84
$N_{60}P_{90}$	266	256	16.18	6.10	100.3	243.4	14.29	69.93
$N_{120}P_{90}$	544	314	16.51	6.98	123.5	350.9	17.56	88.01
$N_{240}P_{90}$	681	372	20.19	7.54	171.1	445.3	15.60	85.67
CD	NS	NS	NS	NS	NS	NS	NS	9.2
$N_{60}S_0$	284	278	14.65	6.78	85.4	255.0	12.18	59.98
$N_{120}S_0$	622	376	15.07	7.46	101.3	316.6	14.46	74.40
$N_{240}S_0$	819	459	19.44	8.15	152.5	415.1	12.87	66.99
$N_{60}S_{45}$	278	252	16.42	6.76	99.6	246.9	14.17	68.49
$N_{120}S_{45}$	508	309	17.22	7.06	121.9	283.7	17.74	75.31
$N_{240}S_{45}$	643	358	20.67	7.58	166.9	388.1	14.09	74.52
CD	72	NS	NS	0.73	NS	NS	NS	NS
P_0S_0	613	390	15.25	7.54	101.2	313.0	12.22	61.58
P_0S_{45}	496	337	18.48	7.90	123.7	285.3	13.16	55.81
$P_{90}S_0$	537	352	17.53	7.39	124.9	344.8	14.12	72.66
$P_{90}S_{45}$	497	275	17.73	6.36	135.2	327.2	17.51	89.74
CD	NS	NS	1.93	0.73	NS	NS	NS	7.51

Addition of each increment of nitrogen showed a significant increase in nitrogen uptake. Application of phosphorus caused an increase in nitrogen uptake from 299 to 336 mg pot^{-1}. Sorghum, being a forage crop responds well to nitrogen application. In an earlier study, Vashishatha and Dwevedi (1997) showed better utilization levels of nitrogen as reflected by crude protein yield of the crop when P was also applied with increase in levels of nitrogen

Uptake of phosphorus, sulphur, potassium, calcium and magnesium

Phosphorus uptake was increased with increasing nitrogen level from N_{60} to N_{120} (Table 1).Further increase in nitrogen dose caused a reduction in phosphorus uptake. Applications of both phosphorus and sulphur caused significant increase in phosphorus uptake. N x P interaction was significant at N_{120} and N_{240} levels. Phosphorus uptake was found to increase to a great extent due to combined application of phosphorus and sulphur and the effect of sulphur was more pronounced when phosphorus was also applied.

Table 2. : Uptake (mg pot^{-1}) of S, K, Ca and Mg by sorghum as influenced by the applications of N, P and S

Treatments	S uptake		K uptake		Ca uptake		Mg uptake	
	35 DAS	70 DAS	35 DAS	70 DAS	35 DAS	70 DAS	35 DAS	70 DAS
N_{60}	15.58	51.65	86.1	313.4	26.56	114.8	10.35	38.81
N_{120}	18.48	59.80	102.8	332.6	28.07	121.0	12.01	43.18
N_{240}	23.00	79.19	116.6	362.9	31.57	150.6	13.50	50.41
CD	3.07	9.30	8.23	37.4	3.38	18.4	1.54	6.18
P_0	17.76	53.46	93.5	291.8	27.49	116.4	11.37	41.59
P_{90}	20.28	73.64	110.2	380.8	29.97	141.2	12.53	46.67
CD	2.51	7.60	6.72	30.6	NS	15.0	NS	5.05
S_0	16.56	58.79	95.3	329.2	28.94	144.6	11.82	48.86
S_{45}	21.48	68.30	108.4	343.3	28.53	113.0	12.09	39.41
CD	2.51	7.60	6.72	NS	NS	15.0	NS	5.05
$N_{60}P_0$	14.70	45.47	79.5	276.5	25.70	111.1	9.88	37.62
$N_{120}P_0$	17.94	51.98	92.1	283.1	27.06	104.2	11.96	41.66
$N_{240}P_0$	20.64	62.92	108.8	315.9	29.73	134.1	12.28	45.50
$N_{60}P_{90}$	16.45	57.84	92.7	350.4	27.42	118.6	10.82	40.00
$N_{120}P_{90}$	19.02	67.63	113.6	382.1	29.08	137.8	12.06	44.69
$N_{240}P_{90}$	25.36	95.46	124.4	410.0	33.41	167.2	14.73	55.32
CD	NS	NS	NS	NS	NS	NS	NS	NS
$N_{60}S_0$	13.24	46.98	79.8	289.5	26.25	135.5	10.76	44.55
$N_{120}S_0$	16.27	53.94	96.5	331.1	27.41	139.1	11.73	48.48
$N_{240}S_0$	20.16	73.47	109.7	367.1	33.15	159.3	12.97	53.55
$N_{60}S_{45}$	17.91	56.33	92.4	337.2	26.86	94.2	9.94	33.07
$N_{120}S_{45}$	20.68	63.67	109.2	334.0	28.73	102.9	12.29	37.88
$N_{240}S_{45}$	25.85	84.91	123.6	358.8	29.99	141.9	14.04	47.27
CD	NS	NS	NS	NS	4.78	NS	NS	NS
P_0S_0	15.21	52.93	87.7	292.8	29.82	134.4	11.84	47.83
P_0S_{45}	20.32	53.98	99.3	290.9	25.17	98.5	10.91	35.36
$P_{90}S_0$	17.91	64.66	102.9	365.8	28.05	154.9	11.81	49.89
$P_{90}S_{45}$	22.64	82.62	117.4	295.8	31.89	127.6	13.26	43.45
CD	NS	10.74	9.5	NS	NS	NS	NS	NS

Sulphur uptake was increased with increasing levels of nitrogen (Table 2). It varied from 11.96 mg pot^{-1} in pots receiving no phosphorus or sulphur and with the lowest level of nitrogen to 28.09 mg pot^{-1} in pots receiving highest dose of nitrogen along with the application of both phosphorus and sulphur at 35 DAS. Individual application of sulphur increased sulphur uptake from a mean value of 16.56 to 21.48 mg pot^{-1}. The effect of phosphorus alone was also found to be significant. Much higher values of sulphur uptake were recorded at 70 DAS as both sulphur content and dry matter accumulation were increased. The values ranged from 44.04 mg pot^{-1} in no phosphorus and sulphur treatments with the lowest level of nitrogen to 107.7 mg pot^{-1} in pots receiving full doses of all the three nutrients. Sulphur uptake was increased with increasing nitrogen levels from N_{120} to N_{240}. Both phosphorus and sulphur showed significant effect in increasing sulphur uptake by sorghum. Combined application of both of these nutrients was more pronounced in increasing uptake of sulphur compared to their individual application.

Potassium uptake was increased with increasing levels of nitrogen up to N_{240} at 35 DAS (Table 2). Application of both sulphur and phosphorus caused a significant increase in potassium uptake individually as well as in combination. The overall values ranged from 71.8 mg pot^{-1}, with N_{60} without the application of phosphorus or sulphur to 127.5 mg pot^{-1} due to N_{240} with the applications of both phosphorus and sulphur. At 70 DAS, more than three-fold increase in potassium uptake by sorghum was obtained compared to that in the first stage. It varied from 313.4 to 362.9 mg $pot^{-1}$and the variation was due to the main effects of nitrogen, phosphorus and sulphur. It followed the similar trend as in the earlier stage. No interaction effect was found to be significant.

Table 3 : Effect of N and P on per cent S derived from fertilizer and per cent fertilizer S utilization by sorghum

	35 Days After Sowing			Mean	70 Days After Sowing			Mean
	N_{60}	N_{120}	N_{240}		N_{60}	N_{120}	N_{240}	
Per cent S derived from fertilizer								
P_0	37.35	45.89	69.38	50.87	12.51	24.65	20.68	19.28
P_{90}	42.87	42.36	38.06	41.09	12.81	9.66	11.24	11.23
Mean	40.11	44.13	53.72		12.66	17.16	15.96	
Per cent fertilizer S utilization								
P_0	7.15	9.81	18.07	11.68	6.51	14.33	14.42	11.75
P_{90}	8.75	10.19	11.69	10.16	9.34	7.66	10.88	9.29
Mean	7.95	9.92	14.88		7.93	10.99	12.65	
CD(P=0.05)		N	P	N x P		N	P	N x P
%Sdff		12.09	9.68	17.11		3.29	2.68	4.65
Per cent fertilizer S utilization		3.36	NS	4.76		3.86	NS	5.46

Calcium uptake was increased with increasing nitrogen levels and effects of all the nitrogen levels were statistically significant at 35 DAS (Table 2). Phosphorus and sulphur applications could not affect calcium uptake. The trend, thus, indicate the reduction in the content of calcium in the initial stage due to increased growth of the crop with the application of these nutrients. At 70 DAS, main effects of all the three nutrients were significant. Increasing levels of nitrogen from N_{120} to N_{240} caused a significant increase in calcium uptake. Phosphorus application also caused more calcium uptake by sorghum. However, it was reduced with sulphur application. Continued absorption of calcium but not to the tune of growth rate of the crop was, thus, evident in reducing the content of calcium but increasing its uptake at the later stage.

Magnesium uptake varied with increased levels of nitrogen only at 35 DAS (Table 2). It was increased from its lowest value (9.01 mg pot^{-1}) recorded in pots receiving the lowest level of nitrogen with sulphur but no phosphorus to the highest level of 10.35 mg pot^{-1} in pots receiving the highest level of nitrogen with both phosphorus and sulphur. At 70 DAS, magnesium uptake increased to a great extent. All the three applied nutrients caused significant variation in magnesium uptake at this stage. Increasing levels of nitrogen increased the values considerably. Phosphorus application also increased magnesium uptake but the values were found to be reduced with sulphur application.

It is evident from the results that increased uptake of plant nutrients also caused better assimilation of nitrogen as reflected by decreased NO_3-N content in plants with application of phosphorus and sulphur. It is especially important for forage crops because nutrient imbalance in diet often aggravates other health hazards like grass tetany or hypomagnesia in cattle. Tetany results when total nitrogen: water soluble carbohydrate ratio was greater than 0.3 and nitrate can be a part of total nitrogen content and contribute to tetany problem (Kemp and Hart, 1957)

Per cent sulphur derived from fertilizer (% Sdff) and per cent fertilizer sulphur utilization

At 35 DAS, % Sdff values ranged from 37.35 to as high as 69.38 (Table 3). The lowest and the highest values were recorded in pots receiving the lowest (N_{60}) and the highest (N_{240}) dose of nitrogen, respectively with no phosphorus application. The difference in %Sdff values due to nitrogen application was significant between N_{60} and N_{240} only. However, phosphorus application caused a reduction in %Sdff values. This reduction was significant at N_{240} level. At 70 DAS, nitrogen application at N_{120} caused increase in % Sdff values and beyond this level of nitrogen application there was no significant effect. Phosphorus application caused a reduction at this stage also. This reduction was significant at both N_{120} and N_{240} levels. Decrease in %Sdff values with application of fertilizer phosphorus could be due to competition between the two anions resulting from similar uptake pathways within the plant (Caldwell *et al*., 1969; Aulakh and Pasricha, 1983).

At 35 DAS, 7.15 to 18.07% fertilizer sulphur was utilized by sorghum. Fertilizer sulphur utilization was increased with increasing nitrogen levels up to N_{240}. Phosphorus application showed no significant effect on it. However, phosphorus application along with the highest dose of nitrogen caused a significant reduction in it. At 70 DAS, utilization of fertilizer sulphur was not appreciably changed from the earlier stage due to either of the treatments, except at N_{240} without the application of phosphorus. The values ranged from 6.51 to 14.42. Nitrogen application from N_{60} to N_{120} caused a significant increase in the values. Increased rate of nitrogen application always require increased uptake of sulphur for better utilization as both nutrients are required for protein formation and their assimilatory pathways are interdependent (Abdin *et* al., 2001). Main effect of phosphorus was not significant but it caused a reduction at N_{120} level. This reduction in fertilizer sulphur utilization might be due to competition between sulphate with phosphate for uptake pathways (Marok and Dev, 1980).

From this study it can be inferred that NO_3-N accumulation in sorghum results from increased rate of nitrogenous fertilizer. The elevated level of nitrate in fodder can be checked to some extent through balanced fertilization of sulphur and phosphorus. Avoiding feeding cattle with the fodder at too early stage may be another measure to check the health hazard.

REFERENCES

Marok, A.S. and Dev, G. 1980. Phosphorus sulphur relationship in Berseem (*Trifolium alexandrium*) as measured by yield and plant analysis. *J. Nuclear Agric. Biol.* **9**: 54-56.

Abdin, M.Z., Ahmad, A., Khan,I., Qureshi, M.I. and Abrol, Y.P. 2001. Effect of sulphur and nitrogen nutrition on nitrogen accumulation and nitrogen harvest in rapeseed-mustard (*Brassica juncea* L and *Brassica campestris* L). In W.J.Horst *et al*.(ed.) Plant nutrition-Food security and sustainable agroecosystems. 816-817.

Johnson, J.L., Schneiderm, N.R., Kelling, C.L.and Doster, A.R. 1983. Nitrate exposure in prenatal beef calves. Amer. Assn. Vet. Lab Diagnosticians. 30th Proceedings, 167-180.

Crawford, R.F.. Kennedy, W.K. and Davidson, K.L. 1965. Physiology of nitrate poisoning in sheep. *Annals New York Academy of Science*, 683-693.

Kemp, A., Geurink, J.H., Haalstra, R.T. and Malestein, A. 1977. Nitrate poisoning in cattle. 2. Changes in nitrate in rumen fluid and methaemoglobin formation in blood after high nitrate intake. *Neth. J. Agri. Sc*. **25:** 51-62.

Vashishatha, R.P. and Dwivedi, D.K. 1997. Effect of nitrogen and phosphorus on 'MP Chari' sorghum (*Sorghum bic*olor). *Indian J. A*gron. **42**(1): 112-115.

Grover, H.L.,Nair, T.V.R. and Abrol, Y.P. 1978. Nitrogen metabolism in upper three leaf blades of wheat at different soil nitrogen levels. 1. Nitrate reductase activity and content of various nitrogenous constituents. *Physoil. Plant*. **42**: 287-292.

Aulakh, M.S. and Pasricha, N.S. 1983. Response of gram and lentil to phosphorus as influenced by sulphur and its residual effect on moong. *Bulletin of the Indian Society of Soil Science.* **12**: 433-438.

Caldwell, A.C., Seim, E.C. and Rehm, G.W. 1969. Sulphur effect on the elemental composition of alfalfa and corn. *Agronomy Journal*. **61**: 632-634.

Jackson, M.L. 1973. Soil Chemical Analysis. Printice Hall India Pvt. Ltd. New Delhi.

Kemp, A. and Hart, M.L.1957. Grass tetany in grazing milking cows. *Neth.J.Agric.Sci*. **5**:4-17.