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Effect of Intercropping of *Sorghum bicolor* cv and *Sorghum sudanese* with Local Variety *Dolicus lablab* on the Level of Hydrocyanic Acid

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Abstract: This study was conducted at Shukaba Livestock Research Station (SLRS) to investigate the effect of intercropping of *Sorghum Sudanese (S. Sudanese)* and *Sorghum bicolor* cv Wad Ahmed (*S. bicolor* cv Wad Ahmed) with *Dolicus lablab (D. lablab)* on the level of hydrocyanic acid (HCN) in the intercropped plants compared to their corresponding mono cropping plants .The result showed that the level of HCN in *S. bicolor* cv Wad Ahmed is significantly ($p<0.01$) higher than in *S. Sudanese* .However, the same trend was found in the *D. lablab* intercropped with *S. bicolor* cv Wad Ahmed compared to intercrop of *S. Sudanese* . The nitrogen content was not significantly different between all plants in the study .While both sulphur and nitrogen to sulphur (N: S) ratio was significantly ($P<0.05$) different. Both . *S. Sudanese* and *S. Sudanese* intercropped with *D. lablab* had significantly ($P<0.05$) higher sulphur and lower N:S ratio than both *S. bicolor* cv Wad Ahmed intercropped with *D. lablab.*

Key words: Sorghum sudanese, Sorghum bicolor, Dolicus lablab, hydrocyanic acid

INTRODUCTION

Cyanide occurs in the leaves of Sudangrass *(Sorghum bicolor*) and sorghum (*S. bicolor*) plants as the cyanogenic glucoside dhurrin (P. hydroxyl-(s)-mandelnoitrite B-D,glucopyranoside).Degradation of dhurrin yields equimolar amount of hydrocyanic, glucose and P-hydroxbenzalybenzaldhyde (P-HB) (Francis[1]. Busk and Moller[2] reported that the cyanide potential is highest shortly after onset of germination. At this stage the nitrogen application has no effect on dhurrin content , whereas in older plants nitrogen application induces an increase. Wheele[3] concluded that the Hydrocyanic (HCN) potential (mean 0.04%) in forage sorghum was increased 28% by nitrogen fertilizer and reduced 34% by phosphorus fertilizer and the ratio of nitrogen to sulphur (N:S) after adjustment for potential loss of sulphur in detoxicattion of HCN was increased from 25:1 to 29 : 1 by nitrogen fertilizer. However, Muldoon[4] who ran an experiment included Sorghum bicolor hybrids, *S. Sudanese*. *S. bicolor* x *S. Sudanese hybrids*, perennial sorghum, maize and pennisetum and Echinochloa millets grown in alkine clay soil recorded that *sorghum* sp. particularly the sweet sorghum hybrids and perennial sorghum had high HCN (0.15 and 0.14), respectively at week eight, they had low content of suplhur especially in the stem fraction and marginally high N:S ratio. The authors concluded that piper sudangrass had low HCN potential which would allow it to be grazed early when digestibility was high (70% at week7). However, maize had negligible HCN potential and N:S ratio below 15:1. The content of the cyanogentic glucoside dhurrin in sorghum varies depending on plant age and growth condition[5]. Busk and Moller[2] found that content of dhurrin correlates well with the activity of the two biosynthetic enzymes CYPIEI and CYP79EI and with the protein and mRNA levels of the two enzymes .The cyanide content increases gradually in the growing plant when it is about 55 cm long to reach a maximum when the length is 80 cm (2.54 mg/100 g)[6].

In the Sudan forage sorghum represent one of main green fodder for ruminants and most of it grown under irrigation specially in summer season. Due to increased need for protein supplementation , for ruminants in SLRS and high cost of urea fertilizer an intercropping programme including both *Sorghum Sudanese* and *Sorghum bicolor* cv Wad Ahmed with *Dolicus lablab* was suggested by department of nutrition and forage production this suggested he present study with the objective of exploring the effect of intercropping of *D. lablab* with *S. Sudanese* and *S. bicolor* cv Wad Ahmed on levels of hydrocyanic acid in sorghum.

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MATERIALS AND METHODS

A study to investigate the effect of intercropping of *Dolicus lablab* (*D. lablab*) with *Sorghum bicolor* cv Wad Ahmed (*S. bicolor* cv Wad Ahmed) and *Sorghum Sudanese* (*S. Sudanese)*on levels of Hydroyanic acid (HCN), was conducted in March 2005 at Shukaba Livestock Research Station (SLRS) (about 10 km south Wad Medani town).

Two feddans (about 0.8 ha) each of *S. bicolor* cv Wad Ahmed, *S. Sudanese*, *S. bicolor* cv Wad Ahmed intercropped with *D. lablab* and *S. Sudanese* intercropped with *D. lablab* were sown on 20th March 2005 for fodder production. Each of single crop (*S. bicolor* cv Wad Ahmed and *S. Sudanese* received 50 kg/fed of urea. While both of the intercropped plants received no urea.

Samples from each single and intercropped plants were collected randomly by throwing steel quadrate for ten times in different directions for ten weeks (starting from week, 1 to week$_{10}$). Mid rib were removed from leaves of each sample. The mid rib free leaves were kept in plastic bags at - 20°C for chemical analysis .

Chemical analysis which were carried out in soil and chemistry laboratory in the Faculty of Agricultural Sciences of the University of Gezira, included dry matter HCN, nitrogen and sulphur determination.

For the release of HCN from the plant samples, enzymatic digestion of plant glucoside by almond seed extract was employed according to Francis[1]. Using the same procedure the release HCN was captured (absorbed) in 0.1N NaOH. To 0.005 N Ag NO_3 , 2 ml of 10% KI and 5-6 ml 6 M ammonium solution were added. The HCN absorbed in NaOH solution was titerated against the Ag NO_3 solution according to Vogel[7]. However, the end point which appears as bluish-while opalescence was seen against a black background.

For sulphur and nitrogen determination plant samples from each two feddans were collected bi-weekly, using the same procedure mentioned previously for samples used in HCN determination.

The sulphur content of the plant samples were obtained according to the methodology described by Ryan[8]. In this methodology plants SO_4-S was extracted with 15% CaCL2 .$2H_2O$ and the measurement of SO4-S concentration in the extract was carried out by a turbidimetric procedure using barium chloride. The turbidity was read in spectrophotometer type Milton Roy at wave length 470. While the dry matter DM and the nitrogen content of each samples was obtained according to AOAAC[9].

Statistical design was randomized block design in a factorial arrangement. Where the factors include 4 treatments x 10 weeks in replicate for HCN and treatments x 5 weeks also in replicate for plant SO_4-S and nitrogen content.

RESULTS AND DISCUSSION

As shown in Table 1 and the Fig. 1 of the results the Hydrocyanic acid Potential (HCNP) in both sorghum species and their *D. lablab* intercropped plants increased during the first 3 and 5 weeks in the intercropped and single crop, respectively (during germination time) after which it decreased with plant age. This result coincided with Busk and Moller[2,6]. However, the same table of the result showed significant ($p<0.05$) differences in HCN-p between the different sorghum species used in this study. This fact is well supported by Muldoon[10] (2 and 3), who studied the sorghum leaf HCN-p in different sorghum varieties. The HCN-p in *S. bicolor* cv Wad Ahmed is significantly ($p<0.01$) higher than in *S. Sudanese* throughout the ten week course of the experiment. While

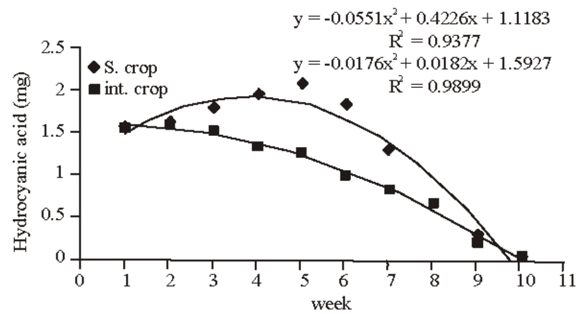

Fig. 1: Corrlelation between time and hydrocyanic acid

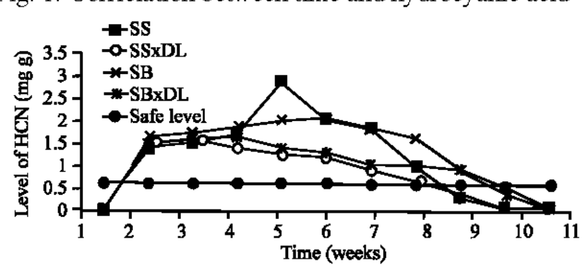

Fig. 2: Safe grazing time for S. Sudanese and S. bicolor cv Ahmed and their D. lablab intercropped

Table 1: Mean HCN-potential mg g^{-1} Dm in different *sorghum* sp. and their *D. lablab* intercropped

Week	*S. Sudanese*	*S. Sudanese xD. lablab*	*Sorghum bicolor*	*Sorghum Biocolorx DL*	Mean
1	1.42	1.54	1.64	1.55	1.54
2	1.52	1.60	1.73	1.60	1.61
3	1.71	1.41	1.88	1.64	1.66
4	2.09	1.27	2.04	1.42	1.66
5	2.06	1.25	2.09	1.30	1.68
6	1.84	0.94	1.84	1.07	1.43
7	0.99	0.67	1.65	1.03	1.09
8	0.29	0.44	1.00	0.94	0.67
9	0.10	0.09	0.54	0.39	0.28
10	0.07	0.03	0.06	0.09	0.06
Mean	1.21	0.92	1.45	1.10	1.17

SE= 0.006.CV.=2.43%

Table 2: Mean nitrogen content g/kg DM in different sorghum species and their *D. lablab* intercropped

Week	*S. Sudanese*	*S. Sudanese xD. lablab*	*Sorghum bicolor*	*Sorghum biocolorx DL*	Mean
1	24.6	24.6	24.5	24.7	24.7
3	22.0	23.0	20.9	20.9	21.9
5	20.0	18.8	18.5	19.1	19.1
7	18.1	18.7	18.1	17.7	18.2
9	17.4	17.9	17.6	17.5	17.6
Mean	20.6	20.6	19.9	20.0	20.3

SE= 1.1.CV.=7.75%

Table 3: Mean sulphur content g kg^{-1} DM in different *sorghum* sp. and their *D. lablab* intercropped

Week	*S. Sudanesex*	*S. Sudanese D. lablab*	*Sorghum bicolor*	*Sorghum biocolorx DL*	Mean
1	1.01	1.01	0.88	0.86	0.94
3	1.01	1.01	0.87	0.87	0.94
5	0.98	0.98	0.91	0.89	0.94
7	0.95	0.96	0.91	0.90	0.93
9	0.92	0.92	0.91	0.95	0.93
Mean	0.97	0.98	0.90	0.89	0.94

SE= 0.008. CV.=1.22%

Table 4: Mean nitrogen to sulphur ratio (N:S)in different *sorghum* sp. and their *D. lablab* intercropped

Week	*S. Sudanesex*	*S. Sudanese D. lablab*	*Sorghum bicolor*	*Sorghum biocolorx DL*	Mean
1	24.7	24.8	24.8	28.0	26.4
3	21.3	21.0	24.7	24.1	22.8
5	19.6	18.3	21.2	20.2	19.8
7	18.6	17.9	20.9	19.9	19.3
9	19.1	18.2	18.9	19.4	18.9
Mean	20.7	20.0	22.8	22.3	21.5

SE= 0.209 CV.=3.08%

in both of the single crops (*S. bicolor* cv. Wad Ahmed and *S. Sudanese)*the HCN-p level is higher than in their corresponding *D .lablab* intercropped plants .Generally there is a negative curvilinear (R2=0.94) and (R2=0.99) strong correlation between the HCN-p and the plant age in single and the intercropped plants respectively. The same relation was reported by AL-Sultan[6] for sorghum Halepanses.

Despite the different sources of nitrogen (urea vers *D. lablab*) used, the result (Table 2) revealed no significant difference in nitrogen content among the different sorghum species and even between each sorghum species and its *D. lablab* intercropped plants. However, the levels of nitrogen content (Table 2) in the present experiment are higher than that of 16.8 g kg DM derived from MAFF[11] for sorghum plants . It was well documented that higher levels of nitrogen increase the level of HCN-p[10]. This may explain the higher levels of HCN-p in these plants of this experiment.

When the sulphur content in different plant samples was detected, the result (Table 3) revealed a significant ($p<0.01$) difference. Both *S. Sudanese* and its *D. lablab* intercropped have a significantly ($p<0.05$) higher SO4-S content than *S. bicolor* cv Wad Ahmed and its *D. lablab* intercropped. While, there was no significant difference in SO_4-S between the *S. Sudanese* and its *D. lablab* intercropped. Also the same trend was found in *S. bicolor* cv Wad Ahmed and its *D. lablab* intercropped .Higher SO4-S content of *S. Sudanese* and *D. lablab* intercropped is reflected on a significantly ($p<0.01$) narrow N:S ratio compared to *S. bicolor* cv Wad Ahmed and its *D. lablab* intercropped (Table 4).

These facts may explain the lower levels of HCN-p in *S. Sudanese* and its *D. lablab* intercropped compared to *S. bicolor* cv Wad Ahmed and its *D. lablab* intercropped. The same findings are well supported by Muldoon[10] who reported that increased sulphur level and decreased N:S ratio decrease the level of HCN-p. Despite the decrease in N:S ratio in both *S. Sudanese* and its *D. lablab* intercropped, this ratio is still higher than the effective ratio of 15:1 reported by Muldoon[4] and 10.1 to 14.3: 1 calculated from ARC[12] for ruminant nutrition . However other factors affecting the level of HCN-p such as phosphorus Wheeler[3] and the dhurrin two biosynthetic enzymes CYPIEI and CYP79EI were not detected in this study.

Generally despite the advantage of earlier decrease of HCN-p in the intercropped sorghum plants, like the single plants could not be grazed safely before week 8 for *S. Sudanese* and its *D. lablab* intercrop while for *S. bicolor* cv Wad Ahmed and its intercropped plants the grazing should be delayed for another week to reach a level (<600 ppm) suggested by Stolenow and Lardy [13] for HCN safety for the grazing ruminants (Fig. 2).

It could be concluded that unless other advantages of intercropping of sorghum with *D. lablab* are needed, both single and intercropped sorghum at least in the present study had the same HCN-p for the grazing ruminants.

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