Current Trends in Natural Sciences
Vol. 8, Issue 16, pp. 55-62, 2019

Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521

Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

HYDROCYANIC ACID CONTENT, FORAGE YIELD AND SOME QUALITY FEATURES OF TWO SORGHUM-SUDAN GRASS HYBRID CULTIVARS UNDER DIFFERENT NITROGEN DOSES IN THRACE, TURKEY

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Current Trends in
Natural Sciences

Abstract
Purpose of the experiment is to research the influence of different nitrogen doses on hydrocyanic acid content, forage yield and some forage quality traits in two sorghum-Sudan grass hybrids (cv. Nutri Honey and cv. Aneto. The experiment was laid out in a two-factor factorial in a randomized complete block design, with three replications. The ½ of the nitrogen fertilizers (0 (control), 40, 80, 120, 160 kg ha^{-1}) were applied sowing and the rest of the nitrogen was given at plants reached 35 cm height. The plants were harvested at three cuts (15 cm from the ground) in year of the field trial at 100 cm plant height. Immediately after cutting, ½ of the nitrogen doses were applied to each plot and the rest of the nitrogen was given when the plants reached 35 cm height. The nitrogen application at the rate of 160 kg N ha^{-1} significantly increased the number of leaves per plant, stem diameter, HCN content, total green and dry matter yields, crude protein ratio over control. The maximum number of leaves per plant (40.69), stem diameter (1.34 cm), HCN content (106.71 mg kg^{-1}), total green fodder yield (13.93 t ha^{-1}), total dry matter yield (4.35 t ha^{-1}) and crude protein ratio (14.23 %) were observed in 160 kg N ha^{-1}. Cultivars crude fiber (28.37 %), acid detergent fiber (30.01 %) and neutral detergent fiber (57.09 %) contents decreased depending on the nitrogen doses. Cultivars 'Nutri Honey' and 'Aneto' were found to be the best choices in terms of crude protein, crude fiber, acid detergent fiber, neutral detergent fiber contents, total green fodder and dry matter yields and benefits when fertilized with nitrogen at the rate of 160 kg ha^{-1} under irrigated same ecological conditions. However, it should be noted that HCN content increases in this dose of nitrogen for green fodder.

Keywords: forage yield, prussic acid, quality traits, sorghum-Sudangrass hybrids

1. INTRODUCTION

The sorghum-Sudangrass hybrids (*Sorghum bicolor* (L.) Moench x *Sorghum sudanense* (Piper) Stapf) are crosses between forage type sorghum (*Sorghum bicolor* (L.) Moench) and Sudangrass (*Sorghum sudanense* (Piper) Stapf). Compared with corn, they have less leaf area, more secondary roots and a waxier leaf surface (Eltelib, 2000). Also they are taller compared with Sudangrass (Bowman et al., 2000).Sorghum-Sudan grass hybrid is one of the most important warm season grasses in semi-arid regions of the world. In recent years, data of atmospheric gases emission have shown that levels of CO_2 are increasing. Many research that the average temperatures throughout the world will rise in the next few decades, resulting in higher global temperatures, affecting rainfall regimes and increasing extreme weather events such as heat waves, droughts, strong winds, and heavy rains. Droughts are damaging because of the long-term lack of water available to the crops,

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especially in semi-arid regions of the world (Ates, 2016). Sorghum-Sudan grass hybrids are a highly drought-tolerant forage crops. It is drought tolerant from corn (*Zea mays* L.). Therefore, recently, sorghum-Sudan grass hybrid cultivars are becoming popular as an annual summer forage option with many desirable features (Mut et al., 2017).

The optimum temperature for it growth is 25-30 °C. Sorghum-Sudan grass hybrids are adaptable to a wide range of soil conditions, but it grows best in loamy and clay-loamy soils. Ideally, soil pH should be in the range 5.5-7.0 for optimum crop development and forage yield. The biomass of sorghum-Sudan grass hybrid is usually used green fodder or for preparation of silage, while it is rarely used for the preparation of hay or grazing (Erić et al., 1999). Depending on use of the biomass (hay, silage, or herbage), suitable agronomic practices are applied (Glamočlija et al., 2011). If these plants are grown for fresh biomass or for grazing, sowing is denser, with higher consumption of seed per hectare, while for silage production, plants are grown on a larger vegetation area (Kruzin and Časovskih, 1997). Plant nutrition is of great importance for obtaining a higher yield and higher nutritive values of the forage crops (Glamočlija et al., 2011). Nitrogen (N) and other macro elements are major limiting nutrients for growth of forage grasses and other forage crops and this explains the improvement in forage yield by external supply of these elements to soils that are deficient in them (Tena and Beyene, 2011). Applications of macro and microelements have a direct effect on the proportion of plant N present as true protein. The N uptake by the plant increases rapidly with the amount of N application and this leads to build-up of nonproteinic organic N, thereby decreasing the proportion of proteinic N with increased amounts of N applied. The effect of N fertilization on the amino acid profile of proteinic N is not fully described. According to the effect of N fertilization on the crude protein (CP) con tent of forage grass, cell wall digestibility may be lowered on less fertilized swards (Peyraud and Astigarraga, 1998). The nitrogen requirement for crop production has traditionally been determined from field experimentation involving different rates of application of nitrogen fertilizer (Muchow, 1998). Mahmud et al. (2003) reported that application of nitrogen increased crude protein, fodder and dry matter yield in forage sorghum.

Cyanogenic glycoside compounds occur in at least 700-800 species of plants, including sorghum-Sudangrass hybrid varieties. Dhurrin, the most important cyanogenic glucoside, in the presence of the enzyme b-glucosidase releases sugar and hydrocyanic acid (HCN), a colorless very volatile liquid, considered one of the most toxic substances ever known. Ruminants are more susceptible to HCN intoxication compared to monogastric. The acidic stomach pH of monogastrics do not allow the enzyme linamarase to act and, therefore, the cyanide release slows down, allowing time for its elimination without reaching the lethal dose (Dowling and Mckenzie, 1993; Ateş, 2012; Simili et al., 2013). The early growth stages of sorghum-Sudangrass hybrid varieties are considered cyanogenic because they contain cyanogenic glycoside, esters that can release toxic substances when the plant structure breaks due to stress caused by grazing, trampling, drought, wilting, freezing, cutting or high N and potassium applications (Montagner, 2005; Simili et al., 2013). The objective of this investigation was to determine the effect of different nitrogen doses on hydrocyanic acid content, forage yield and some quality traits of two sorghum-Sudan grass hybrids (cv. Nutri Honey and cv. Aneto) in Thrace, Turkey.

2. MATERIALS AND METHODS

The research was carried out in 2014-2015 on clay loam soil with pH 6.9 on the farm land Hanoglu/Muratli-Tekirdag in Turkey located at 41.0 °N, 27.0 °E, about 121 m altitude sea level,

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with a typical subtropical climate.The average rainfall of this area is 16 to 40 mm and average summer temperature is 21.53 °C.
The experiment was laid out in a two-factor factorial in a randomized complete block design, with three replications. Two varieties (cv. Nutri Honey and cv. Aneto) were used in the experiment. The varieties was sown in plots of 10 rows with a spacing of 35 cm and 5 m in length (Ateş, 2012). The net plot size is 10.50 m^2.At each year, the seedswere sownat a rate of 80 kg ha^{-1} (Avcioglu et al., 2009) during the second week of May and irrigation applied just after sowing and then irrigation was done after 12-15 days. The water supply required for the growing of sorghum x Sudangrass hybrids were fulfilled by irrigation because of inadequate precipitation during the growing season (May–September). ½ of the nitrogen fertilizers (0 (control), 40, 80, 120, 160 kg ha^{-1}) were applied sowing and the rest of the nitrogen was given at plants reached 35 cm height. Ammonium nitrate as a source of nitrogen is used in the experiment. At each year, a basal fertilizer containing phosphorus (70 kg ha^{-1}) was incorporated into the soil at the time of land preparation. The plants were harvested at three cuts (15 cm from the ground) in year of the field trial at 100 cm plant height. Immediately after cutting, ½ of the nitrogen doses were applied to each plot and the rest of the nitrogen was given when the plants reached 35 cm height.
The number of tiller per plant, number of leaves per plant, leaf/stem ratio and stem diameter (cm) were determined at ten plants (at 100 cm plant height) which is randomly. The stem diameter was measured with a between third and fourth node. Total green fodder yield (t ha^{-1}) (in each year, at three cuts)was obtained in 2 m^2 quadrates, and calculated. Total dry matter yield (t ha^{-1}) for those samples were calculated by drying approximately 500 g samples at 55 °C for 48 hour followed by storage for a further day at room temperature (Ates and Tekeli, 2007).
Dry plant samples were ground to small (≤1 mm) pieces and used for the analyses. The samples were analyzedfor HCN by the Guignardtest (AOAC, 2007). Then, the total HCN content in mg kg^{-1}were calculated by the formula described by Bradbury et al. (1999) as follows:
Total HCN contents (mg kg^{-1}) =396 x absorbance reading.
The samples were analyzed for N using procedures of the Association of Official Analytical Chemists (AOAC, 2007). Crude protein (CP) content (%) of the samples were calculated by multiplying N contents by a coefficient of 6.25. The crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents (%) were determined by Weende and Van Soest methods (AOAC, 2007; Van Soest et al., 1991). All samples were analyzed in triplicate. All data were analyzed statistically by analysis of variance using MSTAT-C software. The percentages were arcsine transformed before statistical analysis to ensure homogeneity of variance. Whenever the interaction with years was not significant, means of two years for treatments were compared using an ANOVA protected least significant difference (LSD) test.

3. RESULTS AND DISCUSSIONS

The different N doses affected the HCN content, number of leaves per plant, leaf/stem ratio, stem diameter, CP, CF, NDF, ADF contents, total green fodder and dry matter yields (Table 1 and 2). Contrary to the CF, NDF and ADF ratios, the HCN content, CP ratio, number of leaves per plant, leaf/stem ratio, stem diameter, total green fodder and dry matter yields increased depending on the nitrogen doses.
The effects of N doses, cultivars and cultivar x nitrogen dose interaction on the number of tillers per plant (4.08-4.15) were not significant (Table 1). The effect of cultivars on leaf/stem ratio was found to be significant (P□0.01), whereas the number of leaves per plant (40.38-41.00) and leaf/stem ratio (0.85-0.88) were influenced significantly by cultivar x nitrogen dose interaction.

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Table 1. The some morphological properties, total green fodder and dry matter yields of sorghum-Sudan grass cultivars (Cv) under different nitrogen doses (ND) (means of two years)

	Nitrogen doses (kg ha^{-1})					
	0	**40**	**80**	**120**	**160**	**Means[¥]**
Varieties	**The number of tillers per plant**					
Nutri Honey	4.08	4.11	4.10	4.13	4.13	4.11
Aneto	4.15	4.12	4.08	4.12	4.10	4.11
Means[†]	4.12	4.12	4.09	4.13	4.12	
LSD	Cv: NS	ND: NS	Cv x ND: NS			
	The number of leaves per plant					
Nutri Honey	25.30h	32.10f	35.36d	36.33c	41.00a	34.02
Aneto	27.00g	33.12e	32.54e	37.74b	40.38a	34.16
Means	26.15d	32.61c	33.95c	37.04b	40.69a	
LSD	Cv: NS	ND: 2.999*	Cv x ND: 0.710**			
	Leaf/stem ratio					
Nutri Honey	0.69d	0.75c	0.80b	0.86a	0.85a	0.79b
Aneto	0.71d	0.80b	0.82b	0.87a	0.88a	0.82a
Means	0.70c	0.78b	0.81b	0.87a	0.87a	
LSD	Cv: 0.029**	ND: 0.051**	Cv x ND: 0.026**			
	Stem diameter (cm)					
Nutri Honey	0.78	0.91	0.98	1.02	1.32	1.00
Aneto	0.80	0.93	0.98	1.10	1.35	1.03
Means	0.79d	0.92c	0.98b	1.06b	1.34a	
LSD	Cv: NS	ND: 0.125*	Cv x ND: NS			
	Total green fodder yield (t ha^{-1})					
Nutri Honey	10.13	11.33	12.47	13.25	13.97	12.23
Aneto	10.29	11.47	12.65	13.20	13.89	12.30
Means	10.21e	11.4d	12.56c	13.23b	13.93a	
LSD	Cv: NS	ND: 0.663*	Cv x ND: NS			
	Total dry matter yield (t ha^{-1})					
Nutri Honey	2.99	3.22	3.89	4.22	4.50	3.76
Aneto	2.93	3.17	3.92	4.19	4.20	3.68
Means	2.96e	3.20d	3.91c	4.21b	4.35a	
LSD	Cv: NS	ND: 0.134*	Cv x ND: NS			

*$P<0.05$, **$P<0.01$, NS: $P>0.01$ and 0.05, [†]The means different nitrogen doses of with different letter for the same row are significantly different. [¥]Sorghum-sudangrass hybrid cultivars means and nitrogen doses x cultivar interactions with different letter of the column are significantly different.

The cultivar 'Aneto' exhibited higher value than other cultivar for the leaf/stem ratio (0.82) (P<0.01). The plant height, main stem length, number of branches per plant, the number of tiller per plant, stem diameter, number of leaves per plant, leaf length and leaf weight are important characters used to determine green fodder yield and dry matter yield (Ate and Servet, 2004); besides, leaf length, leaf weight, leaf/stem ratio, protein, fiber and mineral contents are important traits for forage quality (Ates, 2011). Moreover, sorghum-Sudan grass hybrids, sorghum and Sudan grass cultivars show good response to nitrogen application. Contrary our results, Lourenco et al. (1993) reported that the application of nitrogen fertilizer up to 100 kg N ha^{-1} increased tiller numbers of Sudan grass. Iptas and Brohi (2002) found that higher nitrogen rates continued to increase the number of tillers per plant rather than the number of plants per unit area. Mustafa

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(1995), who investigated the effect of nitrogen fertilizer dose and time of application on the growth and yield of four sorghum hybrids, reported application of nitrogen, has little effect on number of leaves per plant. Simili et al. (2013) investigated growth rate of sorghum x Sudan grass hybrid during fall and reported a leaf/stem ratio 0.5 for its.

Table 2. The HCN content and some quality traits of sorghum-sudangrass cultivars (Cv) under different nitrogen doses (ND) (means of two years)

	Nitrogen doses (kg ha^{-1})					
	0	**40**	**80**	**120**	**160**	**Means**
Varieties	**HCN content (mg kg^{-1})**					
Nutri Honey	66.71	70.13	85.74	95.56	105.67	84.76
Aneto	65.98	70.45	84.94	96.00	107.75	85.02
Means†	66.35e	70.29d	85.34c	95.78b	106.71a	
LSD	Cv: NS	ND: 2.733*	Cv x ND: NS			
	CP (%)					
Nutri Honey	10.90	11.20	12.33	13.41	14.27	12.42
Aneto	10.88	11.45	12.50	13.39	14.18	12.48
Means	10.89d	11.33d	12.42c	13.40b	14.23a	
LSD	Cv: NS	ND: 0.651**	Cv x ND: NS			
	CF (%)					
Nutri Honey	32.17	31.50	30.78	29.88	28.41	30.55
Aneto	31.98	31.25	30.54	29.74	28.33	30.37
Means	32.08a	31.38b	30.66c	29.81d	28.37e	
LSD	Cv: NS	ND: 0.598**	Cv x ND: NS			
	NDF (%)					
Nutri Honey	61.23	60.55	59.12	58.11	57.20	59.24
Aneto	61.45	60.21	58.98	58.00	56.98	59.12
Means	61.34a	60.38b	59.05c	58.06d	57.09e	
LSD	Cv: NS	ND: 0.886**	Cv x ND: NS			
	ADF (%)					
Nutri Honey	35.41	35.03	33.66	31.74	30.05	33.18
Aneto	35.00	34.89	33.49	31.84	29.97	33.04
Means	35.21a	34.96b	33.58c	31.79d	30.01e	
LSD	Cv: NS	ND: 0.235**	Cv x ND: NS			

*$P<0.05$, **$P<0.01$, NS: $P>0.01$ and 0.05, †The means different nitrogen doses of with different letter for the same row are significantly different.

160 kg N ha^{-1} application significantly increased HCN (106.71 mg kg^{-1}), CP (14.23 %), stem diameter (1.34 cm), total green fodder (13. 93 t ha^{-1}) and dry matter (4.35 t ha^{-1}) yields compared to other doses(Table 1 and 2). The main criteria for the evaluation of the nutritional value are the increase of digestible matter and the decrease of lignin and HCN content for fodder sorghum, Sudan grass and their hybrids. Nitrogen used in plant nutrition had no influence on increasing the total digestible substance content in dried biomass samples. Fodder sorghum, Sudan grass and their hybrids are plants with a strong root system and good suction power, and they are well capable of using unused nitrogen salts necessary for the nutrition of preceding crops (Glamočlija et al. 2011). Hydrocyanic acid content is heritable and subjected to modification through selection and breeding, as well as by climate, stage of maturity, stunting of plant, type of soil and fertilizer (Khatri et al., 1997). Nitrogen application is considered essential for growth and regrowth during growing season.

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However, higher level of nitrogen application may increase HCN contents of forage sorghum; ultimately poisoning animals (Aziz-Abdel and Abdel-Gwad, 2008; Sher et al. 2012). Wheeler et al.(1990) studied the sorghum-Sudan grass hybrids and reported strong influence of nitrogen fertilization and plant age on the HCN contents in the leaves. Bahrani and Ghenateghestani (2004) reported an increase of 55% higher HCN content with nitrogen application in forage sorghum. Sher et al. (2014) found the fertilizing sorghum forage with 120 kg N ha^{-1} produced the highest HCN content (21.5 and 13.4 mg/100 g) at booting and 50 % heading stage, respectively while the lowest HCN content of 17.8 and 11.0 mg/100g was determined in control treatment where no fertilizer was applied. They reported the increase of HCN content (21-22%) was more with the application of 120 kg N ha^{-1} when compared with control treatment (0 kg N ha^{-1}). Similarly, Wheeler et al.(1980) revealed 28% increase in HCN by applying nitrogen fertilizer. Aziz-Abdel and Abdel-Gowd (2008) reported that increase in nitrogen application resulted in enhanced HCN in sorghum. Afzal et al.(2012) reported that application of urea increased the fresh and dry weight of multicut sorghum. Mut et al. (2017) determined that the hay yield ranged between 7.67 and 8.31 t ha^{-1} among Sudan grass and sorghum-Sudan grass hybrids. Total green fodder and dry matter yields of sorghum species determined in the present research was similar to Mut et al. (2017).
Data regarding CF (28.37 %), NDF (57.09 %) and ADF (30.01 %) ratios in sorghum-Sudan grass hybrids have lowest ratios by applying N at the 160 kg ha^{-1} (Table 2). Forage grasses are higher in NDF, ADF and ADL at a given stage of growth than forage legumes. The quality of forage crops is best estimated by their potential dry matter intake and dry matter digestibility, which are determined by the NDF and ADF fractions, respectively. Both NDF and ADF increase as the plant matures causing a decline in the quality of the forage (Linn and Kuehn, 1997; Ates, 2016). Effects of different nitrogen rates on hay yield and some quality traits of Sudan grass and sorghum x Sudan grass hybrid varieties were studied by Gulumser and Mut (2016), who reported the average plant height, leaf ratio, hay yield, crude protein ratio, crude protein yield, ADF and NDF ratio were ranged from 200.9–205.5 cm, 36.02–39.90 %, 1.39–2.55 t da^{-1}, 8.17–11.90 %, 113.5–304.9 kg ha^{-1}, 37.51–40.31 % and 62.59–67.34 % in the 2013-2014 respectively.Mut et al. (2017) reported that NDF and ADF contents in sorghum-Sudan grass hybrids ranged from 67.27 to 67.62 % and 36.55 to 36.73 %, respectively. The results were similar to those reported by these researchers.

4. CONCLUSIONS

From the results of two years field experiments, Nutri Honey and Aneto cultivars were found to be the best choices in terms of CP, CF, ADF, NDF contents, total green fodder and dry matter yields and benefits when fertilized with nitrogen at the rate of 160 kg ha^{-1} under irrigated same ecological conditions. However, it should be noted that HCN content increases in this dose of nitrogen for green fodder.

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Current Trends in Natural Sciences (on-line)
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ISSN-L: 2284-9521
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ISSN: 2284-9521
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