

THE EFFECTS OF DIFFERENT SOWING TIME AND HARVESTING HEIGHT ON HYDROCYANIC ACID CONTENT IN SOME SILAGE SORGHUM (*Sorghum bicolor* L.) VARIETIES

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ABSTRACT

The objective of this experiment was to determine the hydrocyanic acid (HCN) levels that have poisonous effects on animals, in 4 different sorghum and 1 sudangrass species, which were developed by Batı Akdeniz Agricultural Research Institute and widely used in our country. Experiments were carried out in experimental fields of institute during 2014 and 2015 growing seasons. Rox, Leoti, Early Sumac and Nes sorghum varieties with Gozde 80 sudangrass variety were cultivated as May-June and June-July planted crops in a randomized block design with three replications. Plants were harvested when they reached 40, 80 and 120 cm heights and they were extracted in order to determine HCN contents. The analysis of variance indicated that there were significant differences in terms of HCN content in all varieties. The HCN levels of varieties were determined in Gozde 80, Early Sumac, Nes, Leoti and Rox in descending order. In the first year, the highest HCN level was determined in Early Sumac variety with 40 cm harvesting height at the first sowing time, the lowest level was determined in Gozde 80 variety with 120 cm harvesting height at the second sowing time. In the second year, the highest HCN level was determined in Gozde 80 variety with 40 cm harvesting height at the first sowing time, the lowest HCN level was determined in Early Sumac variety with 120 cm harvesting height at the second sowing time. At the end of the experiment, the highest HCN content was found at the first sowing time with 40 cm harvesting height.

Key words: Harvesting height, Hydrocyanic acid, Silage sorghum, Sowing time

INTRODUCTION

Sorghum is one of the most used five main products in human and animal nutrition in the world, and it is in fifth place after wheat, corn, rice and barley in terms of cultivation and production (Baytekin et al., 1996). Total sorghum harvesting area 33,1-44.9 million hectares in the world in 2013-2014 years respectively (FAO, 2017). Sorghum is widely used as a silage crops, because it can be harvested more than once during the vegetation period (Tüsüz et al., 1984). Additionally, it is more resistant to drought and diseases than maize (McGinty, 1972) and shows a better development in high temperatures (Okuyucu, 1975).

The Mediterranean region is suitable for the cultivation of various forage crops due to the soil structure and climate. In the researches for the Mediterranean region, it was proved that different harvesting stages had an effect on some silage parameters of sorghum. It is reported that sorghum and sudangrass are more economical than maize as roughage source in the coastal region of Antalya by the reason of they can be harvested at least 3 times, and they have similar nutritional value with maize. Sorghum can be planted as main and second

crop, planting time show the differences year by year according to the climatic conditions, therefore especially silage quality, plant development and plant nutrient contents show differences (Oten and Cakmakçı, 2010). There have been several studies about sorghum in Turkey. In studies, hay yield was determined 1273.5 - 2184.8 kg da⁻¹ by Mulayim et al. (2009), 3683-4791 kg da⁻¹ by Tugay (2009), 1908.98 - 2343.41 kg da⁻¹ by Karadas (2008), 1654 kg da⁻¹ by Cecen et al. (2005), 1453.40-1975.60 kg da⁻¹ by Yılmaz and Hosaflioglu (2000) and 1050 - 1423 kg da⁻¹ by Soya (1999). Forage yield of sorghum was determined 5355 - 8173 kg da⁻¹ by Gul and Baytekin (1999), 7000-10000 kg da⁻¹ in irrigated conditions and 1500 kg da⁻¹ in arid conditions by Açıkgöz (2001). Despite of the high yield potential and high tolerance in different regions and climatic conditions, under unsuitable conditions, sorghum and sudangrass contain different ratios of HCN (Hydrocyanic acid / Cynaide / Prussic Acid), which is a toxic substance. These unsuitable conditions can be exemplified as heavy nitrogen fertilization, drought, acidic soils, phosphorus deficiency in the soil, low heat and frost. If the plant is exposed to these unsuitable conditions, it accumulates nitrate in abnormal rate in the stem. Afterwards, Nitrate

becomes toxic for animals by transforming into nitrite. Thus, more than 0.5% nitrate in dry matter is dangerous. Hydrocyanic acid does not exist in large quantities in the plant as long as plant is vigour. Plant cells contain glycosides (dhurrin) and enzymes (emulsion) separately during growing period. When the plant cells are damaged by harvesting or grazing, glycosides and enzymes are combined and transformed into HCN as a result of chemical reaction. The highest amount of glycoside is found in young plants and leaves, and HCN concentration decreases when plant height increases and maturation of plant progresses. HCN level decreases to the lowest level at the beginning of flowering for sudangrass, and at the milk stage for silage sorghum. Considering these toxic substances reduce when sorghum and sudangrass grow up, plant height should be 70-100 cm for grazing or harvesting (Acikgoz, 2001; Haque and Bradbury, 2001; Wheeler et al., 1990; Tusuz et al., 1984; Langer and Hill, 1982;). Isakov and Basova (1973) reported that young leaves and plants contains more HCN than old leaves. Moreover, while different amounts of nitrogen treatments increase the level of HCN 50-180 ppm, phosphorus deficiency and low temperatures increase the HCN level. Aziz-Abdel and Abdel-Gwad (2008) and Pholsen and Somsungnoen (2004) indicated that, while nitrogen fertilization increases the rate of HCN level, it reduces the sugar rate. Williams and James (1983) determined that herbicide treatment (2,4-D and 2,4,5-T) increases the level of HCN in sorghum. Lang (2001) stated dangerous levels of HCN concentrations of fresh forage as <150 ppm is low, 150-200 ppm is medium and >200 is high for livestock (Table 3). Koch and Paisley (2012), stated dangerous levels of HCN in forages; less than 600 ppm is not toxic, 600-1800 ppm is potentially toxic and over 1800 ppm is very toxic. The aim of this study was investigation of HCN level in different growth stages of silage sorghum whether reach to harmful level for animals, and determination of the difference among varieties in terms of the HCN level. Moreover, the effect of first and second sowings and different harvesting heights on HCN levels were observed.

MATERIALS AND METHODS

The study was conducted in the experimental areas of Batı Akdeniz Agricultural Research Institute between 2014 and 2015. The experimental field was under Mediterranean climate that has hot and dry summers, and mild and rainy winters. The climate data of experimental area, maximum, minimum and average temperatures and rainfall for 2014, 2015 and long time were shown Table 4. The land where the research was conducted, have silty with clayey, saltless, very high limey, strong alkaline and low organic matter content soil. Rox, Gozde 80, Leoti, Early Sumac and Nes sorghum varieties, which are widely used in the Mediterranean region and registered by

BATEM, were used as plant material. The study was established in a randomized block design with three replications. In the first year, May-June and June-July planted crops were sowed in 07.05.2014 and 05.06.2014 respectively. In the second year May-June and June-July planted crops were sowed in 06.05.2015 and 07.06.2015 respectively. Sorghum seeds were sown in 3 m long plots with 8 rows. Row spacing and intrarow spacing were 45 cm and 10 cm respectively. Seeds were sown 2 kg per decare. In this experiment, 4 kg da⁻¹ 21 % ammonium sulfate and 8 kg da⁻¹ 42 % triple super phosphate were applied with sowing in both years. Any fertilizer treatments were not done throughout the growth season. Weed control was done manually in both years. The total rainfall received during the crop growth period (May-July) was 26.2 and 51.1 mm during 2014 and 2015, respectively. Irrigation was done in first sowing time in 08.06.2014, and second sowing time in 01.07.2014 for the first year. Furthermore, it was done in first sowing time in 05.06.2014 and second sowing time in 30.06.2014 for the second year. After that, irrigation was not done until the end of the experiment. All other agronomic practices were done throughout the growing seasons. Plants were harvested at 40, 80 and 120 cm heights and extracted by compression machine. Harvesting times were shown in Table 5. HCN levels in the samples were determined using the colorimetric method that is suggested by Lambert et al. (1975) and adapted by Pirincci and Tanyıldızı (1994). According to this method; calibration curve was prepared using by 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 2, 3, 4, 5, 6, 8, 10 and 20 ppm potassium cyanide solutions (*Sigma-Aldrich Cat. No:31252-100 g*). 1 ml water samples, 1 ml N-chlorosuccinimide-succinimide solution (10 g succinimide *Sigma- Aldrich Cat. No: 59381-500 g* was dissolved in 200-300 ml distilled water, afterwards, 1 g N-chlorosuccinimide *Sigma- Aldrich Cat. No: 109681-100 g* was added and the solution was completed to the 1 lt with distilled water) and 1 ml barbituric acid-pyridine solution (3 g barbituric acid *Sigma-Aldrich Cat. No:185698-25 g* was dissolved in 10 ml distilled water, afterwards, 15 ml pyridine *Sigma-Aldrich Cat. No:360570-100 ml* was added and the solution was completed to the 50 ml with distilled water) were mixed and dropped to the 25 ml flasks, afterwards, the solution was completed to the 25 ml with distilled water. The solution was held on 15 minutes in the darkness for the colour formation. After that, HCN levels of the samples were determined as ppm by observation of absorbance in 575 nm in the spectrophotometer (Shimadzu, UV 1600) (Figure 1.). Obtained data in the experiment, was evaluated with analysis of variance by SAS (1998) statistical software. The differences between means were compared according to Duncan's multiple range test (Duzgunes et al., 1987).

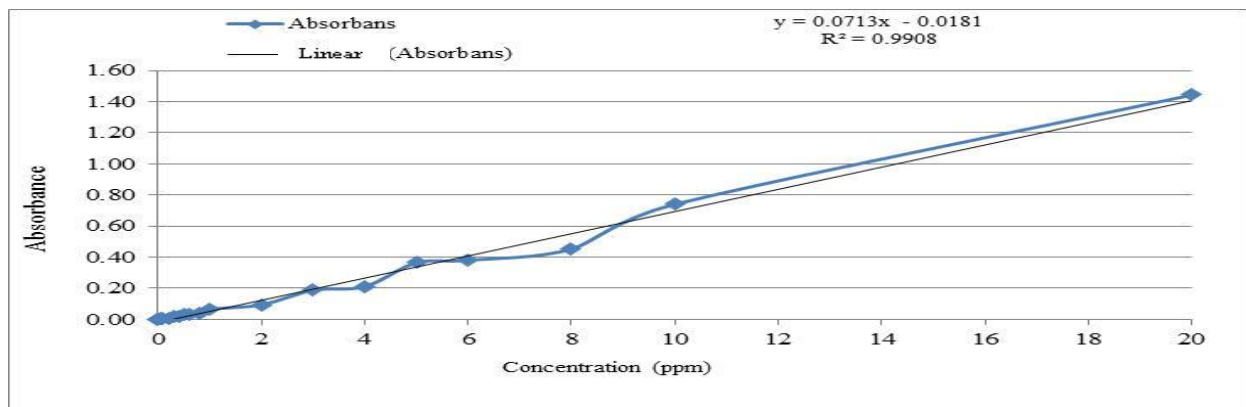


Figure 1. Calibration curve

RESULTS AND DISCUSSION

The analysis of variance for combined years showed that the variance was highly significant for HCN content, among the investigated characters. According to the results of analysis of variance at the 0.01 level; there was a significant difference between years, varieties, sowing times and harvesting heights. Moreover, there was a significant difference between year*variety, year*sowing time, year*harvesting height, variety*sowing time*harvesting height, year*variety*sowing time*harvesting height interactions (Table 1). Duncan test for means of HCN levels, which were obtained from using materials in the experiment, was shown at Table 2. It was observed that Gozde 80 sudangrass had the highest value with 8.26

ppm among the other varieties which had statistical difference in terms of HCN levels, and it was followed by Early Sumac with 6.60 ppm, Nes with 5.63 ppm, Leoti with 5.29 ppm and Rox with 5.17 ppm. When means of HCN levels were examined according to sowing time; while HCN level was high with 7.36 ppm at the first sowing time, it was 5.02 ppm at the second sowing time. When HCN level was observed in terms of harvesting height, the average amounts were determined respectively as 8.99 ppm, 5.66 ppm, 3.93 ppm while the harvesting heights were 40 cm, 80 cm, 120 cm. By the reason of statistically differences between HCN levels in both years, the years were analyzed separately and grouped among themselves (Table 2).

Table 1. Variance analysis of HCN levels (Combined Years)

Source of variation	d.f.	Sum of squares	F value
Year	1	793.6740050**	4148.75
Blok(Year)	4	0.3076000 NS	1.61
Variety	4	59.4092256**	310.55
Year*Variety	4	27.3094411**	142.75
Sowing time	1	247.5968450**	1294.25
Year * Sowing time	1	27.5264006**	143.89
Harvesting Height	2	351.8564850**	1839.25
Year*Harvesting Height	2	22.6833450**	118.57
Variety*Sowing Time*Harvesting Height	22	8.1375691**	42.54
Year* Variety*Sowing Time*Harvesting Height	22	10.6688556**	55.77
Error	116	22.191333	0.19

** Significant at 0.01 probability level. NS Non Significant

In the first year, while Early Sumac variety had the highest HCN level at the first sowing time with 40 cm harvesting height, Gozde 80 variety had the second highest HCN at the first sowing time with 40 cm harvesting height. Gozde 80 variety had the lowest level of HCN at the second sowing with 80 cm and 120 cm harvesting heights. Leoti, Nes and Rox varieties had 9.72

ppm, 5.69 ppm, 5.50 ppm HCN levels respectively at the first sowing time with 40 cm harvesting height. While Gozde 80 variety had the highest HCN level with 6.62 ppm at the second sowing time with 40 cm harvesting height, HCN levels of Early Sumac, Rox, Nes and Leoti varieties were 4.61 ppm, 4.32 ppm, 2.41 ppm and 2.35 ppm respectively (Table 2).

Table 2. HCN content (ppm) of five silage sorghum varieties at the different sowing time and cutting heights

Years	Sowing time	Harvesting height	Varieties					Mean of Year	CV
			Gozde 80	Early Sumac	Nes	Leoti	Rox		
2014	First Sowing Time	40	12.89 b	14.15 a	5.69 e	9.72 c	5.50 e	8.29 a	
		80	5.35 e	4.57 fg	4.40 g	2.80 i	3.69 h		
		120	3.48 h	4.79 f	2.82 i	2.13 k	2.86 i		
	Second Sowing Time	40	6.62 d	4.61 fg	2.41 jk	2.35 jk	4.32 g		
		80	1.17 lm	3.58 h	1.39 l	1.43 l	2.76 i		
		120	0.96 m	2.57 ij	1.22 lm	1.27 lm	1.27 lm		
2015	First Sowing Time	40	15.43 a	12.67 b	11.25 cd	10.37	10.77	7.05	
		80	10.60 d	10.47 d	8.16 f	6.87 gh	7.95 fg		
		120	7.86 fg	6.92 gh	7.31 fg	4.30 ij	5.20 i		
	Second Sowing Time	40	14.83 a	7.75 fg	11.19 cd	9.46 e	7.82 fg		4.09
		80	11.68 c	4.76 ij	7.53 fg	7.75 fg	6.25 h		
		120	8.25 f	2.41 k	4.16 ij	5.11 i	3.73 j		
Mean of Variety			8.26 a	6.60 b	5.63 c	5.29 d	5.17 d		
Mean of Harvesting Height			40 cm: 8.99 a		80 cm: 5.66 b		120 cm: 3.93 c		
Mean of Sowing Time			1 st Sowing Time: 7.36 a			2 nd Sowing Time: 5.02 b			

Table 3. Safe Limits of HCN Concentration in Forage

Relative degree of toxicity	Concentration of HCN in dry forage		Concentration of HCN in fresh forage	
	ppm	mg HCN/100 gm	ppm	mg HCN/100 gm
Very low	--	0-25 (0-0.025%)	--	--
Low	0-500	25-50 (0.025-0.050%)	<150	<15
Medium (potential)	500-700	50-75 (0.050-0.075%)	150-200	15-20
High (dangerous)	>750	>75 (>0.750%)	>200	>20

*(Lang, 2001)

Table 4. Climate Data (2014- 2015-Long Period)

Years	Climate data	Months		
		May	June	July
2014	Precipitation (mm)	27.2	0.0	0.0
	Mean Temp. (°C)	20.2	25.3	27.5
	Max Temp. (°C)	25.9	32.1	33.1
	Min Temp.(°C)	14.3	18.0	21.3
2015	Precipitation (mm)	46.0	5.0	1
	Mean Temp. (°C)	21.3	23.8	27.7
	Max Temp. (°C)	34.5	38.2	41.3
	Min Temp.(°C)	11.0	15.6	16.4
Long Period	Precipitation (mm)	31.8	7.9	3.0
	Mean Temp. (°C)	20.5	25.4	28.4
	Max Temp. (°C)	38.0	44.8	45.0
	Min Temp.(°C)	6.7	11.1	14.8

Table 5. Harvesting Time

Years	Varieties	First Sowing Time			Second Sowing Time		
		Harvesting height (cm)			Harvesting height (cm)		
		40	80	120	40	80	120
2014	Gözde 80	18.06.2014	25.06.2014	01.07.2014	11.07.2014	17.07.2014	24.07.2014
	Nes	11.06.2014	18.06.2014	26.06.2014	04.07.2014	11.07.2014	21.07.2014
	Leoti	19.06.2014	26.06.2014	04.07.2014	14.07.2014	21.07.2014	29.07.2014
	Rox	11.06.2014	17.06.2014	25.06.2014	07.07.2014	14.07.2014	21.07.2014
	Early Sumac	11.06.2014	18.06.2014	25.06.2014	04.07.2014	10.07.2014	18.07.2014
2015	Gözde 80	15.06.2015	23.06.2015	30.06.2015	08.07.2015	14.07.2015	19.07.2015
	Nes	10.06.2015	16.06.2015	24.06.2015	02.07.2015	09.07.2015	19.07.2015
	Leoti	17.06.2015	24.06.2015	30.06.2015	14.07.2015	19.07.2015	26.07.2015
	Rox	10.06.2015	15.06.2015	23.06.2015	06.07.2015	12.07.2015	19.07.2015
	Early Sumac	10.06.2015	15.06.2015	22.06.2015	01.07.2015	07.07.2015	15.07.2015

In the second year, while Gozde 80 variety had again the highest levels of HCN at the first sowing time with 80 cm harvesting height and at the second sowing time with 40 cm harvesting height, Early Sumac had the lowest amount of HCN with 120 cm harvesting height. At the first sowing time, Early Sumac, Nes, Rox and Leoti had 12.67 ppm, 11.25 ppm, 10.77 ppm and 10.37 ppm HCN levels respectively with 40 cm harvesting height. At the second sowing time, HCN levels were 14.83 ppm in Gozde 80, 11.19 ppm in Nes, 9.46 ppm in Leoti, 7.82 ppm in Rox and 7.75 ppm in Early Sumac with 40 cm harvesting height (Table 2). Studies for determination of the hydrocyanic acid amount in silage sorghum; Haque and Bradbury (2001) reported that while the HCN content was 740 ppm at a week after the germination stage, it reduced to 160 ppm, after three weeks. Zahid et al. (2012) observed HCN contents 10 days, 40 days and 70 days after germination and, 6 hours, 12 hours and 18 hours after harvesting in three commonly grown sorghum varieties and one local variety. 10 days after germination HCN level was 723 ppm, 40 days after germination 209 ppm and 70 days germination it was 126 ppm. Furthermore, in local sorghum variety, 10 days after germination while HCN level was 723 ppm, 6 hours after harvesting 723 ppm, 12 hours after harvesting 658 ppm, and 18 hours after harvesting it was 637 ppm. As a result, HCN levels were very high at early growth stages of all varieties, however it reduced at following growth stages. In addition, Sarfraz et al. (2012) determined HCN levels of the varieties, which were used in the experiment, varied between 255.4 ppm (F-9601) and 346.6 ppm (Hegari). Satpal Duhan et al. (2015) determined HCN content between 761.0 and 170.3 ppm. AL-Sultan (2003) determined HCN content as 0.698 ppm when the plant height was 46 cm, 2.54 ppm when it was 80 cm, and 0.042 ppm when it was 120 cm.

Shaug (1990) stated that HCN level was 0.1 - 55.9 ppm in sorghum bicolor lines, and it was 8.6 - 47.4 ppm in sudangrass at the flowering period. Sotomajor-Rios and Torres-Cardona (1984) were found lower HCN level at 60th day than 45th day in their study, which was about agronomic performance, HCN and heterosis of hybrid

sorghum silage. Furthermore, they identified 191 ppm HCN level in the male parents, which had a high yield potential. Torres et al. (1983) studied about HCN potential and agronomic performance of single and triplet hybrids of silage sorghum, and they observed that HCN level was more than 200 ppm (250-285 ppm), which is a toxic for animals. In this study; it was observed that the amount of HCN was high at the early stages of plant growth and it was low at the following growth stages. Similarly, Haque and Bradbury (2001), Zahid et al. (2012), AL-Sultan (2003) and Sotomajor-Rios and Torres-Cardona (1984) stated that HCN level reduces in parallel with plant growth. HCN levels were different among varieties in this experiment, similarly Zahid et al. (2012) determined the difference among genotypes. HCN levels of the varieties used in this experiment were similar to HCN levels in the studies of Shaug (1990) and AL-Sultan (2003), and they were lower than in the studies of Haque and Bradbury (2001), Zahid et al. (2012), Satpal Duhan et al. (2015), Torres et al. (1983) and Sotomajor-Rios and Torres-Cardona (1984). The reason of the difference between the values, the difference of the genotypes and ecological conditions can be considered. At the same time, it can be said that physiological development period of the plant, temperature, drought, drugs and nitrogen fertilization, etc. had an effect on HCN content of sorghum (Tusuz et al., 1984; Langer and Hill 1982; Acikgoz 2001).

In different studies for determination of the HCN levels in sorghum, it was emphasized that nitrogen manner increased the HCN level (Sher *et al.*, 2012; Bahrani and Ghenatagehstani, 2004; Wheeler et al., 1980; Abdel-Gowd. 2008), however, in this study this situation cannot be demonstrated due to no fertilizer treatment was done. Similarly; Vickery et al. (1987), and Wheeler et al. (1984) reported that temperature and precipitation effect on level of HCN, in this study there was no precipitation during vegetation period, and no relation was found between temperature and HCN level. Irrigation was done only before the first harvesting, therefore, irrigation effect on HCN level could not be determined. For the future studies, the effect of irrigation and climatic conditions on HCN content should be done.

The results of this study showed that the HCN levels in all silage sorghum and sudangrass varieties, in May-June and June-July planted crops and in all harvesting heights, were under the dangerous limit. There were statistically significant differences between the varieties in terms of HCN level. HCN levels were higher in the May-June planted crops than in the June-July planted crops, moreover, HCN levels was high at the beginning of the growth stage and it reduced in parallel with plant growth. It was observed that sorghum and sudangrass varieties, which were used in this experiment, did not contain HCN in a dangerous level for animals, and Leoti and Rox varieties had HCN in a lower levels. Moreover, all varieties had lower HCN level in 120 harvesting height and in June-July planted crops.

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