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HAZARDS OF TOXIC HYDROCYANIC ACID (HCN) IN SORGHUM AND WAYS TO CONTROL IT : A REVIEW

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Abstract

Sorghum is one of the fifth feeding products for human and animal. It is suitable for agriculture in different environmental conditions, which contributes to expanding its production**.** However, the fact that sorghum contains hydrocyanic acid (HCN) to toxic levels for animals in some plant stages or growth conditions makes reduce of expanding in its production. There is not enough knowledge of how to manage this crop and know the factors that lead to an increase in the proportion of HCN to the deadly limits of livestock**.** Studies have shown that early cutting with a duration of less than 35-40 days and environmental stresses during growth stages, as well as excessive nitrogen fertilizer or a lack of phosphorous element in the soil and grazing animals in the early stages of growth or feeding them on new plant leaves are all factors that lead to increase the content of vegetative part of HCN to the dangerous concentrations. Therefore, the cutting time should be delayed, the environmental stress should be avoided, a balanced fertilization system should be adopted and the conditions for safe grazing should be followed.

Keywords: Sorghum, animal poisoning, Prussic acid

Introduction

Sorghum bicolor (L.) Moench is a grain and forage crops important locally and globally. It is used as a grain or green fodder to feed livestock and poultry as palatable hay and silage for animal, because it has high percentage of protein and carbohydrates; it can be used as hay or silage. Sorghum is one of crops with high potential for growth and branching after mowing, which provides more number of shoots during the summer season, as well as low fiber content, and it is described as annual crop with high yield and good quality (Banks, 2005). It is able to tolerate stress of drought and temperatures and grow in the tropical areas as a summer crop, with different types of agricultural soils (Ottman and Olsen, 2009 and Prakash *et al.*, 2010).

This has increased the feeding value of sorghum over the years compared to the value of grains. But the main factors limiting the use of sorghum as a feed is its production of cyangenetic glycoside (which produces hydrocyanic acid HCN) which reduces its nutritional value due to its toxic effects on livestock feeding, as digestive enzymes break down the compound into hydrocyanic acid (HCN). All varieties of sorghum, Leaves and stems contain hydrocyanic acid (HCN) or prussic acid. Also some other plants produce hydrocyanic acid, but in smaller quantities while in sorghum in large quantities that pose a threat to animal species.

Hydrocyanic acid

Hydrocyanic acid (HCN) is a colorless transparent liquid with very toxic vapor and a distinct smell similar to the bitter almond smell lighter than air and rapidly dissipates, its boiling point is 25.6 centigrade, and molecular weight is 27.0253 g /mol. hydrolyzes of Hydrocyanic acid is slowly to give ammonium format.

This acid is not found free in Natural and healthy plants. Rather, it is found in a sugar body called Cytogenic Clocoside, that contains cyanide (CN^-) which converts to Prussic acid when it is digested by specific enzymes (AL-Dulimi, 2012). All plants that follow sorghum genus are distinguished by containing alkaloid substances (glucosides) when they are hydrolyzed, they give a toxic substance that is HCN, which, when absorbed into the animal's body in sufficient quantities, leads to its death (Shafqaf and Al-Dabbabi, 2008). Therefore, it is important to consider the problem of the presence of this acid when growing sorghum.

The effect of HCN on animals

Sorghum can contain both cytogenic so-called dhurrin and digestive enzymes such as beta-glycosidase. if plant tissue is damaged by freezing, cutting, or chewing, the enzymes will be released and become in contact with sugars and produce Prussic acid (Allison and Baker, 2002), bacteria that are endemic in digestive tract of ruminant animals (cows and sheep) can free the aforementioned acid from sugars, and once the cyanide is absorbed, it quickly travels in the bloodstream to all parts of animal's body (Montagner *et al.*, 2005). Non-ruminants (horses and pigs) are less likely to be poisoned with Prussian acid because the enzymes that release HCN are destroyed by lowering the pH in the mono stomach.

Cyanide is characterized by its high toxicity to animals, as it can reach toxic levels of up to 200 micrograms^{-1} g of dry weight, which is the minimum in sorghum leaves, and it can lead to infection of livestock quickly and doses of not less than 0.5 g are sufficient to kill a cow (Carlson and Anderson, 2013), as it interacts with the Cytochrome Oxidase enzyme, which is an important enzyme in the electron transport chain necessary for the cells to use oxygen, and hence will be an inert complex, so cyanide works to inhibit the release of oxygen from the hemoglobin molecules in the blood of the animal, and without oxygen it will be stopped The cellular respiration process which It leads to rapid cell death due to asphyxia (Jadav *et al.*, 2019).

Some sorghum varieties contain relatively high concentrations of cyanide ranging from 400 to 900 mg^{-1} kg. The upper new leaves contain a higher concentration of acid compared to the lower leaves. As for the recent growths, they are much more dangerous than the old ones, especially the growths formed after the plants were exposed to freezing, which the animal have eaten with great desire (Yunus, 1993). Nóbrega *et al.* (2006) concluded that goats feeding on sorghum (halepense L.) 30 days after planting showed severe shortness of breath and frequent urination in animals, a sign of acute HCN poisoning that leads to death. The safe concentration of HCN in green fodder for livestock is 500 ppm based on fresh weight and 200 ppm on dry weight (Karthika and Kalpana, 2017). In general, there are many factors that can lead to cyanide poisoning in cows and sheep. For this reason, it is difficult to apply safe grazing for all conditions. However, as general rule of cyanide levels above 600 mg^{-1} show a grazing danger but the level to 200 mg^{-1} kg still shows threat to very hungry animals (McCuistion, 2017).

Methods for estimating HCN in plants

Several methods have been developed to determine the total content of cyanogen (total cyanide) in sorghum. There are methods for estimating HCN in plant tissues, but they need three basic steps (Nahrstedt *et al.*, 1981)**:**

1. Hydrocyanic acid (HCN) release from plant tissue.
2. Isolation and concentration of the released HCN in order to remove other substances.
3. Estimating the concentration of isolated acid using chemical or physical reaction.

The most important methods used to estimate total cyanide content in plant tissues can be as follows:

1- Enzymatic degradation method
2- Acidic hydrolysis method
3- Combined enzymatic hydrolysis method.

The most commonly used method for estimating hydrocyanic acid concentrations for plants, especially forage crops, is methods of Gorz *et al.* (1977) and Haskins *et al.* (1987) which are performed according to the following steps**:**

1- When taking the plant sample from the field, consideration must be given to preserving the moisture of the sample immediately after cutting by storing it in bags of wet cloth until it reaches the laboratory in order to prevent the loss of part of the moisture by evaporation that leads to the loss of part of the acid.
2- Vegetable parts were cut into very small pieces and a random sample weighing 20 gm. was taken from them and placed in a 150 ml test tube.
3- 10 ml of distilled water is added to the test tube, after which the tube is closed with a tight cork seal and placed in the Autoclave for 30 minutes at a temperature of 120 ° C.
4- the samples are placed in a cold water bath and after cooling an amount of solution is taken and diluted with a standard NaOH solution (0.1N) and then placed in a Spectrophotometer on a wave length of (330nm) to estimate the concentration of HCN acid as referred by Gorz *et al.* (1977) Using the following equation:

$$HCN = \frac{A \times DF \times VE \times 27.03}{Fr\,Wt \times 27.9}$$

A = absorbed amount of diluted base in wave length (330 nm).

DF = dilution constant obtained from the dilution of prepared sample.

VE = volume of distilled water (ml) used for plant tissue extraction.

27.03= molecular weight of HCN (mg / m ml).

Fr Wt. = fresh weight of the extracted tissue.

27.9 = absorption coefficient (ml / m ml) at a wave length (330nm) in a standard solution of NaOH (0.1N)**.**

The concentration of hydrocyanic acid (HCN) can be estimated using the standard curve of the colored complex, according to what is referred by Craigmill (1981), according to the following steps:

1- As samples are taken from the leaves of young plant to preparing the known concentration, by taking 1.4 g of sodium carbonate and 1.4 g of sodium cyanide in 100 ml distilled water containing 1.4 ml of bicaric acid, then it turns color into a brilliant red.
2- Prepare the concentrations 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mol^{-1}, after which the intensity of the resulting color is measured by using the optical density with a spectrophotometer (visible-UV) at a wavelength. 520 nanometers.
3- Draw the calibration curve between the concentrations and the absorbance of the red complex solution, (Figure 1).
4- Keeping the samples in wet cloth bags until they reach the laboratory in order to prevent the loss of part of the moisture by evaporation that leads to the loss of part of the acid.
5- The leaves are cut into small pieces with a dimension of 0.5-1 cm and a weight of 0.2-0.3 g. They are placed in a test tube and added 2-3 chloroform.
6- The guide colored sheet is fixed in a tightly tested tube to prevent acid leakage. After 23 hours of incubation, the guide sheet is raised after changing its color from yellow to a brilliant red then washed in 20 ml of distilled water and readings are taken with a spectrophotometer at a wave of 520 nanometers.

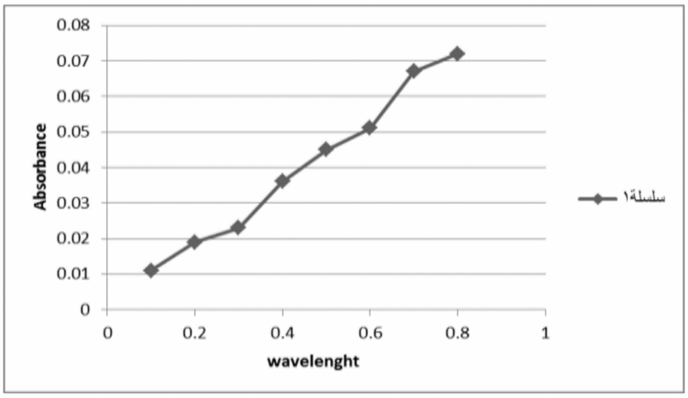

Fig. 1 : Standard curve between concentrations and absorbance of red complex (Dawood and Aboud, 2017)

HCN relationship to plant parts and age

Muthuswamy *et al.* (1976) estimated HCN content of five hybrids of sorghum at different growth stages, as they found that the HCN content in the vegetative part was very high (650 mg kg^{-1} fresh weight) after 18 days of planting and gradually decreased to its lowest concentration (7 mg 1 kg^{-1} fresh weight) after 49 days of planting, while the root part content was somewhat fluctuating if it was low (375 mg^{-1} kg fresh weight) 18 days after planting and then reached its highest level (575 mg^{-1} fresh weight) after 27 and 30 days after planting and then decreased again to reach its lowest content (300 mg^{-1} kg fresh weight) after 53 days of planting (Table 1).

The plant content of HCN was estimated by Chaturvedi (1994) at the flowering and ripening stages with 30 genotypes of sorghum, samples were collected at two stages: the first after 65 days of planting (flowering) and the second after 100 days (seeds ripening). Genotypes were significantly in HCN content in both stages. The concentration of HCN decreased significantly after 65 days of planting. In a study conducted by Al-Rubaie (1995) in which he found a significant effect of the cutting stages in the concentration of hydrocyanic acid, as the stage of vegetative growth outperformed and given highest concentration of 336.9 parts per million, while the stage 100% flowering gave the lowest concentration of 201.7 parts per million.

Table 1 : HCN Content of sorghum hybrids for different growth stage and vegetative part (vegetative and root system) (Muthuswamy *et al.*, 1976).

Days of planting	HCN Content (Mg kg $^{-1}$ fresh weight)	
	Root system	Vegetative system
18	375	650
20	425	600
23	500	575
27	575	300
30	575	200
34	500	150
40	325	75
45	400	43
49	350	7
53	300	15

Srinivasa *et al.* (2006) showed that HCN content of sorghum increased in the early growth stage, which decreased as the plant matured. The HCN content of immature sorghum is relatively higher, which has decreased significantly with plant growth as the HCN content has decreased to a non-toxic level after 45 to 50 days of planting in most types of forage crops and sorghum (Pandey *et al.*, 2011) (Research 2)˙ In a study conducted by Al-Fahdawi (2011), it was found that cutting dates significantly affected the HCN concentration of plant, as the plants of the pasta phase of the grains gave the lowest concentration, while the plants of the vegetative growth stage gave their highest concentration. Simili *et al.* (2013) found that the HCN content of sorghum leaves decreased with the age of the plant, as they ranged between 205.0 and 230.3 mg of HCN / 100 gm dry weight after two weeks of planting and then reached 5.9 and 6.1 mg HCN / 100 g dry weight In the fifth week of life for both the first and second years of experience. The risk of HCN toxicity decreases with plant maturity. Old plants and leaves contain the lowest concentration of toxic HCN (Carlson and Anderson, 2013). Oten (2017) showed that the highest HCN content is for harvested plants with a height of 40 cm compared to cutting with 80 and 120 cm heights in which the latter gave the lowest HCN content. In an experiment conducted by Jadav *et al.* (2019), it included cattle feeding with sorghum (*Sorghum vulgare*) since it reached 15 days after planting until it reached 60 days after planting and a two-week interval between one batch and another during which the quantitative HCN concentration (mg/100 g dry matter) was estimated at each cutting stage, they found that the most appropriate and safe stage in which the animal is fed without any toxic effect is 25% flowering. The sorghum crop is also distinguished by the ability to cutting it more than once by planting and harvesting it after the seeds ripening and then restoring its vegetative growth again from the basal buds. This is what is known as Raton sorghum (Cheyed and AL-Mohammedi, 2016; Cheyed *et al.*, 2014). In this case, the plants will remain in the field until the next season, and the HCN content of the plants will be reduced by up to 27% compared to the plants resulting from seeds planting directly in the second season (El. Fahdawi *et al.*, 2020). The reason for this may be due to the increase in the growth rate of the Raton plants and their early cutting compared to the plants that result from direct seed planting (Cheyed and AL-Mohammedi, 2016).

The relationship of HCN to genotype

Sorghum leaves and stems contain borax acid-sugar, and cannabis is perhaps the least containing the aforementioned acid. The cannabis and sorghum hybrids contain a moderate concentration of borax acid (Shcneider and Andrson, 1986).

Chaturvedi *et al.* (1994), when studying 25 genotypes of sorghum, indicated that 20 of them contain high HCN concentrations in the flowering stage and reached 606 mg^{-1} and are unsafe for animal feeding, while they found that only five genotypes are suitable for feeding Animals where HCN levels reached 230 mg^{-1} kg.

Mohanraj *et al.* (2006) found to study the effect of six genotypes of sorghum and the extent of the resulting hybrid inheritance (24 hybrids) from those genotypes in inheriting the hydrocyanic acid content after 32 days of planting, as they found a significant difference between those genotypes and their hybrids and found that the susceptibility of coalition Special (SCA) for plant content of HCN is higher than its variation in general coalition susceptibility (GCA), and it has been possible to reduce the proportion of hydrocyanic in plants by adopting repeat selection of genotypes that recorded the lowest HCN content and that this trait is influenced by the non-added gene. This result is similar to that of Khatri *et al.* (1997) in a previous study**.**

Iyanar (2001) indicated an expression of the characteristic of plant content of HCN as referring to the act of an additional gene. Mohanraj *et al.* (2006) and ELObeid *et al.* (2006) found a significant contrast between sorghum genotypes in their HCN content. Several studies also confirmed the existence of a discrepancy between the genotypes of the sorghum in its content of HCN and those studies showed that the genetic factor has a significant impact on the determination of the HCN concentration in the plant (Abusuwar and Hala, 2010 and Pandey *et al.*, 2011˒ Sarfraz *et al.* 2012˒ Khan *et al.* 2013 Oten, 2017).

The relationship of HCN to nutrients

Sorghum is a good source of fodder in normal conditions, but when plants are exposed to stress or nutrient imbalance in the soil, this may lead to an increase in the plant content of hydrocyanic acid (HCN) and may reach the deadly level of animals (Sanmugapriya *et al.*, 2017).

Forage sorghum is characterized by its great need for nitrogen fertilizer to produce green fodder, but high quantities of nitrogen fertilizer cause an increase in the concentration of borax sugar in the plant, especially in soils that suffer from a lack of phosphorous (Shcneider and Anderson, 1986). Forage sorghum grown in high soil N and

P had a higher HCN content than in hybrid varieties (Pandey *et al.*, 2011). Aziz-Abdel and Abdel-Gwad (2008) found that increasing nitrogen fertilizer levels lead to an increase in the content of sorghum leaves from HCN. Oten (2017) indicated an increase in soil nitrogen content and a decrease in phosphorous component leading to an increase in the content of sorghum leaves from HCN.

The relationship of HCN to environmental stress

Large quantities of dhurrin may be produced quickly when the sorghum are under the influence of environmental stresses (drought and frost) and when leaf tissues are disrupted (Mohanraj *et al.*, 2006) and increased soil acidity (Oten, 2017). The amount of the dhurrin content responsible for the production of HCN in sorghum leaves varies depending on the genotype, but it is also influenced by the environmental conditions. (Pushpa *et al.*, 2019). The different genotypes of sorghum showed a change in their HCN content when growing in different environments, as it was noted that they were affected by climate factors, plant growth stage, soil type, quantity and quality of added fertilizers (Khatri *et al.*, 1997).

The nitrogen component is one of the major and essential elements that the plant needs throughout the growth. However, a high nitrogen level may increase the Prussian acid content in sorghum plants, increasing the possibility of animal poisoning (Aziz-Abdel and Abdel-Gwad, 2008).

The plant density and the quantity of added fertilizers greatly affect the Prussian acid concentration in the plant, as Sher *et al.* (2012), when studying the effect of three rates of sowing (75, 100 and 125 kg h^{-1}) and three levels of nitrogen (0, 60 and 120) kg h^{-1}) in the sorghum content of HCN that increased nitrogen levels gradually increased the production of HCN regardless of crop variety and growth stage, as nitrogen fertilization treatment showed 120 kg h^{-1} increased in HCN of 64% compared to the control treatment, which indicates that high doses of nitrogen fertilizers showed a threat to the quality of feed produced from sorghum in dry areas.

Sanmugapriya *et al.* (2017), when studying the effect of planting distances (30 x 10, 30 x 15, 30 x 20 and 30 x 25 cm) in the content sorghum of HCN, found that the 30 x 10 planting distance gave the lowest average plant content of HCN. In a study by Pushpa *et al.* (2019) to see the effect of water stress on the content of five genotypes of HCN, it was concluded that the production of hydrogen cyanide (HCN) was more severe in plants growing under drought conditions compared to plants grown under normal irrigation conditions.

Safe feeding for livestock with sorghum

Sorghum is often grown as a direct food source for livestock during the summer or early winter. To avoid animal cyanide poisoning, a careful management system for sorghum crop or its residues must be used when presenting it as forage.

Conditions for safe grazing of sorghum (McCuistion, 2017).

1- Monitoring the new growth that comes out of the harvested plant stems during the summer or from the dry plant bases when the crop passes through environmental conditions that help the growth of those branches that have a higher HCN concentration compared to mature plants. Therefore, animals should not be grazed, especially ruminants (cows, sheep and goats) because they will be more affected compared to non-ruminants (horses). Therefore, grazing should be prevented until these branches progress and HCN concentrations are reduced.
2- The new leaves of the plant have a higher concentration of HCN than the old leaves and stems. As for the grains, there is no HCN. Because the animals are selective in their feeding and looking for young leaves, this causes more accumulation of HCN. It is possible to cutting the apical growth of the plant and leaves them for a period of not less than a week until the concentrations of HCN decrease and then the animals can be left for grazing. This is the case for sorghum that is exposed to freezing in the winter. They must be left for at least a week before the cattle are allowed to graze the fodder crop for the same reason.
3- Avoid grazing hungry or stressed animals on sorghum plants containing the upper limits allowed of HCN, as they will be more greedy and susceptible to absorbing the HCN, so they should be fed with dry fodder such as straw or grains before converting them to grazing because this will reduce their consumption of plants in Pasture.

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