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Effect of Foliar Application with Thiamin B1 on Plant Content of Hcn (Hydrocyanic Acid) of Ten Sorghum Cultivars (*Sorghum bicolor* L. Moench)

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ABSTRACT

A field experiment was conducted at experimental farm, college of Agriculture, University of Anbar, Abu Ghraib (Alternative Site) during the summer season 2016 in silty clay loam soil to study the effectof foliar application with thiamin B1 on plant content of HCN (hydrocynic acid) of ten sorghum cultivars, the layout of experiment was randomized complete block design (RCBD) with three replicates , the first factor consisted of ten cultivars of sorghum (Inkath, Rabih, Kafir, Mabrouk, Buhooth 70, Syrian cultivar, Babil , Warka, Lilo and Ishtar),the second factor was three concentrations of vitamin B1 (thiamine), The plants was cut three timesduring growth season at 50% flowering to estimate the plant content of HCN, the results of this experiment are summarized as follows: the concentrations of HCN in the cultivars samples was safe levelswhen the three cuttings operationsat 50% flowering, Ishtar cultivar gave the lowest concentration of HCN was 93.3, 103.6, 118.4 ppm, at three cuttings respectively, In addition,spraying of vitamin B1with 75 $mg.L^{-1}$concentration gave the lowest average content ofHCN 99.1, 107.8 and 119.7 $mg .L^{-1}$at three cutting respectively , the interaction between the study factors had a significant effect on plant content of (HCN) at the three cuttings.

Keywords: sorghum, thiamine (vitamin B1), hydrocynic acid, cutting.

INTRODUCTION

Livestock is a very important sector in the world, where a large portion of the world's population feeds on it. Most of the world allocates large areas for growing forage crops to increase feed production, which is reflected in animal production because most of the world's population depend on its products, so most countries in the world allocate large areas to grow forage crops to increase the production of forage crops, which is reflected positively on animal production.

The need ofgreen forage is increasing especially in the summer where the lack of rain, high temperatures and salinization of the soil, all these non-biological stresses lead to a severe scarcity of feed during the summer in most parts of the world,sorghum is a very important crop because itstolerance the environmental stress also because it give high feed yield compared to other crops as well as It gives several cuttings during the summer(Olsen and Ottman, 2009 and Prakash, 2010).

There is a problem associated with the cultivation of this crop and most of the species belonging to the genus Sorghumbecause these plants contain alkaloids (glycosides) when hydrolyzed gives a toxic substance is thehydrocynic acid HCN, which, when absorbed in the body of the animal in quantities, leads to the death of these animals so should pay attention to this problem when cultivating white maize, especially in the vegetative parts When used as feed at an early stage of growth (Shafshik and Al-Dababi, 2008), in addition, non-biological stress increases the concentration of HCN in the green forage of the sorghum crop, this causes poisoning of animals that feed on this crop, the sorghumcultivars differ in their content of HCN, which is the main determinant of green forage production of this crop, the grain varieties are characterized by their high concentration of this acid, unlike the feed varieties, which are characterized by low concentration of HCN acid,also the plant leaves contain the most concentration of HCN compared with other plant parts.

In a study conducted by Singh et al. (2010) found that the genotypes (HC308, CSV15 and SU1080) differed significantly in HCN content, where the genotype SU1080 gave the lowest content ofHCN 84.74 and 35.53 ppm when plants werecut after 40 and 60 days of planting respectively. Sheret al (2012) noted thegenotypes different of sorghum in their HCN content, the genotype JS-2000 gave the lowest HCN content at 50% flowering 21.1 $mg.kg^{-1}$, whereas the local cultivargave the highest concentration of acid 49.1 $mg.kg^{-1}$.

When cultivating and producing forage, attention should be given to the selection of appropriate cultivar for feed production and its content of acid (HCN) is low because the plant content of this acid is genetically and can be modified by selection and breeding, the increase in production of green forage requires high amounts of nitrogen fertilizers and this excessive use leads to increased plant content of this HCN as well as the lack of nitrogen fertilizer in all regions and the difficulty of moving from region to another (Karthika and Kalpana, 2017).

It is necessary to look for alternatives to use of nitrogen fertilizers, these alternatives includevitamins, Thiamin is one of these important vitamins and safe in the environment and have no side effects on animal health and food product, Alberta (2015) noted that vitamins are not created by animals because they cannot Although they are very essential in animal feed, so animals depend on plants to obtain them, as well as that the components of food such as proteins, carbohydrates and salts are not enough to grow, there must be additional materials necessary for life, including vitamins (Daoodi, 1990).

The genotypes that are used in breeding programs for the development of are genotypes and new cultivars production characterized by high green feed yield, good quality and low HCN content, Studies should be carried out to develop these genotypes and choose the best to improve their forage qualities and reduce their HCN content.

Thisstudy was conducted to determine the performance and response of ten cultivars of sorghum to spraying with vitamin B1 and importance of effect of interaction between the study factorson plant content ofhydrocynic (HCN) acid and possibility of developing varieties with a lower content of HCN.

MATERIALS AND METHODS

A field experiment was conducted at experimental farm, college of Agriculture, Alanbar University, Abu Ghraib (Alternative Site) during the summer season 2016to study the effect of genotypes (10 genotypes as shown in the table 1) and foliar application with thiamin B1(0, 75, 150 mg.L^{-1}) on plant content of HCN (hydrocynic acid) ,the layout of experiment was randomized complete block design (RCBD) with three replicates, three cuttings taken during the growth season.

Table 1. The genotypes used in the study

Numbers	Genotypes	Codes
1	Inkath	V1
2	Rabih	V2
3	Kafir	V3
4	Mabrouk	V4
5	Buhooth 70	V5
6	Syrian cultivar	V6
7	Babil	V7
8	Warka	V8
9	Lilo	V9
10	Ishtar	V10

The soil was plowed, modified and divided intoexperimental units , the unit area 9 m2 dimensions (3 x 3) m, theexperimental unit contained six lines, the length of each lines 3m. the distance between them 50 cm and between holes 5 cm, the length of each lines 3m, random samples were taken from the experiment soilbefore planting at a depth of 0-30 cm to estimate some physical and chemical properties of soil, the chemical analysis was carried out in the laboratories of the Agricultural Research Department (Table 2).

Table 2. some physical and chemical properties of experimental soil

physical and chemical properties	Values
pH	7.9
EC (dS.m^{-1})	3.5
Total N (%)	0.31
available P (ppm)	13
available K (ppm)	188
Sand (%)	14.7
Silt (%)	55.0
Clay (%) %	30.3
texture	Silty clay loam

The soil was fertilized with phosphate fertilizers before the planting with 100kg (P).ha^{-1} while The Urea fertilizer (46% N) was added at planting as a first dose and the second dose after 20 days of emergence, planting was on 28/3/2016, the experimental units were 90 experimental units resulting from the compatibility between the study factors and three replicates. the solution of thiamin was sprayed for the concentrations of thiamine according to the concentrations used in this study (control treatment was distilled water only) after 50 days of planting in the first cuttings,while the second and third cuttings were sprayed after 30 days of cutting by using knapsack sprayer (15 L capacity)after adding the cleaning fluid (zahi) 15ml.100L^{-1} to reduce the surface tension of the water and to ensure the complete wetness of the plant leaves, Three cuttings were taken during the growth season when the plants reached 50% flowering to study the plant content of this acid according to the following method:

Determination of HCN (ppm):

Hydroxyanic acid (HCN) was estimated in the whole plant by cutting it early at morning and taking the samples and keeping it in wet cloth bags until move it to laboratory to prevent any lossesof moisture by evaporation which leads to loss part ofacid, then cut the samples into small pieces according Haskins (1987),so the whole plant (leaves and stems) were cut into very small pieces and a random sample of 20 g was taken and placed in a test tube, then add 10 ml of distilled water, close tightly with cork cover then put it in Auto clave 30 minutes at a 120℃ temperature,samples were removed and placed in a cool water bath and taking amount of leachate solution and diluted in NAOHNormality 0.1N.

The samples were read by using a UV spectroscopy device with 330nm wavelength according to Corz et al. (1977), after that, hydrodynamic acid was estimated according to the following equation:

$$\text{HCN (ppm)} = \frac{A*DF*VE*27.03}{FrWt*27.9}$$

A = absorption amount of alkaline diluted in the wavelength (330nm).
DF = the dilution factor obtained from dilution of the model prepared for measurement.
VE = distilled water (ml) that used to extract plant tissues.
27.03 = Weight formula of HCN (mg / m mole).
FrWt = wet weight (gm)of extracted tissue.
27.9 = absorption factor (ml / m mole) at the wavelength (330nm) in Normality (0.1N) of Naoh solution.

RESULTS AND DISCUSSION

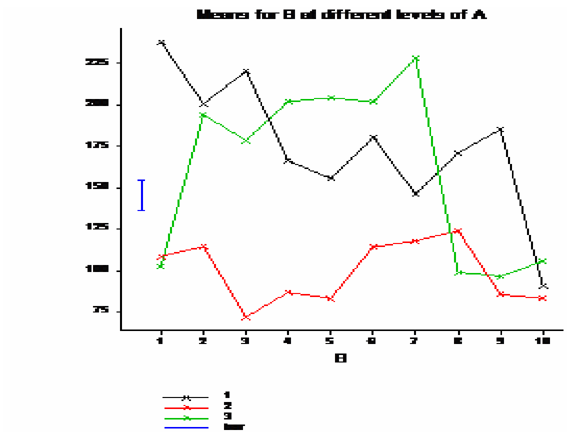
The content of the hydrocynic acid (HCN) in plants of first cutting:

The data of Table 3 and Figure 1 show that the genotype V10 was superior, with the lowest HCN concentration 93.3 ppm, while the 2V genotype gave the highest HCN concentration 169.8 ppm and did not differ significantly with the other five genotypes (V1, V2 , V3, V4, andV5). it may be due to the difference of genotypes according to the difference in theirgenetic differences.

Table 3. Effect of thiamine levels on the HCN concentration of ten sorghum genotypes at the first cutting.

Genotypes	Thiamin concentration mg.L^{-1}			Mean
	0	75	150	
V1	238.0	108.7	102.7	149.8
V2	200.7	114.7	194.0	169.8
V3	220.3	72.0	178.7	157.0
V4	166.3	87.0	202.0	151.8
V5	155.7	83.3	204.3	147.8
V6	180.3	114.3	201.7	165.4
V7	146.3	118.0	228.3	164.2
V8	171.0	124.0	99.0	131.3
V9	185.3	85.7	96.7	122.6
V10	91.0	83.3	105.7	93.3
Mean	175.5	99.1	161.3	21.14
L.S.D		17.93		
		37.05		

The levels of thiamin had a positive effect in reducing level of HCN in the plant, this is a positive indicator, thethiamin level of 75 mg.L^{-1} gave the lowest concentration of HCN 99.1 ppm while the control treatment gave the highest HCN concentration 175.5 ppmthe reason may be due to role of thiamin in improving vegetative growth without need of nitrogen that contributes in increased concentration of acid in the plant, as well as its role in reducing the non-biological stresswhich are exposed to the plant in the summer as well as the role of vitamins in strengthening the immune system in plants and animals (Alberta, 2015 and Smith, 2015), while the interaction between the genotypes and thiamin levels was significant in its impact on this character, interaction treatment (genotype3V x thiamine 75 mg.L^{-1}) gave lower concentration of HCN with decrease percentage by 69.74% compared with interaction treatment (genotype V1 x control treatment).



Content of second cutting plants of hydrocynic acid:

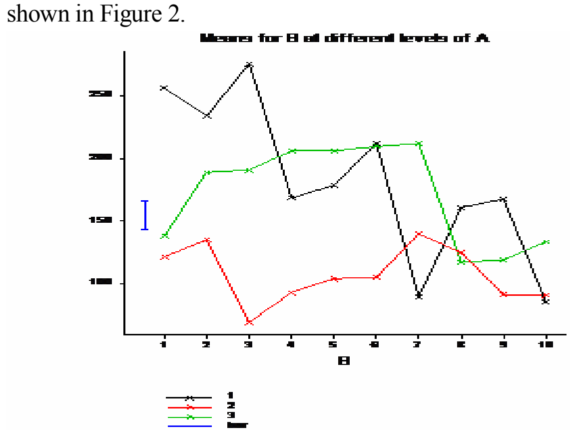
The results of Table (4) showed that the sorghum genotypes was significantly different in HCN concentration, the genotype V10 gave HCN concentration 103.6 ppm, with a decrease by 41.89% compared the V1 genotype which gave the highest concentration of HCN186.1 ppm,It may be due to the difference of genotypes according to differences in their genetic differences, this is in agreement with results of (Fahdawi, 2011 and Pandey et al., 2011), they noted that all species belonging to the genus of Sorghum contain hydrocynicacid but with different concentrations as well thedifferences between the leaves and stems in their content of HCN, the results also showed that the level of 75 mg.L^{-1}of thiamin gave the lowest concentration of HCN was 107.8 ppm while the concentration 0 (control treatment) gave the highest HCN concentration 182.9 ppm.

Table 4. Effect of thiamin levels on the HCN concentration of ten sorghum genotypes at the second cutting.

Genotypes	Thiamin concentration mg.L^{-1}			Mean
	0	75	150	
V1	234.0	135.3	189.0	186.1
V2	256.3	121.7	138.3	172.1
V3	275.3	69.7	191.0	178.3
V4	168.7	93.3	206.0	156.0
V5	178.7	104.3	206.0	163.0
V6	212.0	105.3	209.7	175.7
V7	89.7	140.0	212.0	147.2
V8	161.0	125.0	117.3	134.4
V9	167.7	91.7	119.3	126.2
V10	85.7	91.3	133.7	103.6
Mean	182.9	107.8	172.2	
L.S.D		12.41		27.20
		45.52		

The effect of thiamine in reducing the concentration of HCN in sorghum may be due to the role of thiamine in improving plant growth and improving performanceof enzymes, including antioxidants, which enhance plant resistance to non-biological stresses to reduce increase HCN concentration but within limited concentrations.

Table 4 shows that interaction treatment(V3 genotype x thiamin 75 mg.L^{-1}) gave the lowest HCN concentration 69.7 ppm and did not differ significantly with other 7 genotypes (V4, V5, V6, V9, V10 and V7) with concentration 75mg.L^{-1} and control treatment respectively, while the V3 genotype gave the highest concentration of HCN with control treatment 275.3 ppm as shown in Figure 2.

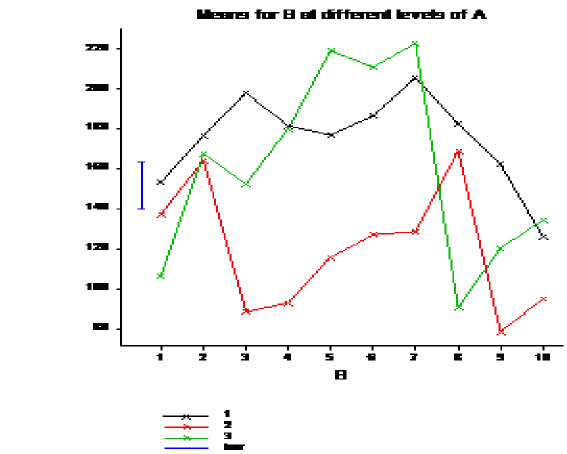


Content of third cutting plants of hydrocynic acid:

Table (5) show there is a significant effect of the genotypes and levels of thiamine and interaction between them in the plant content of the HCN, the genotype V10 gavelowest concentration of HCN 118.4 ppm, with a decrease in HCN concentration by 36.27%compared with genotypeV7 genotype, which had the highest HCN content 185.8 ppm, the difference among genotypes in HCNconcentrations may be due to geneticdifferences among them, the results showed that the thiamin level 75 mg.L^{-1} gave the lowest HCN concentration 119.7 ppm while the control treatment (distilled water only) gave the highest HCN concentration 175.0 ppm, this indicates that thiamin B1 has an effective role in reducing HCN concentration, also the interaction between the genotypes and thiamin levels, the results of Table 4 indicate that interaction treatment(V9 genotype x thiamin 75 mg.L^{1})gave a lowest HCN concentration 78.3 ppm compared to the highest HCN concentration of 223.0 ppm obtained from interaction treatment (7V genotype x Thiamine 150 mg.L^{-1}), that did not differ significantly with 5 other genotypes as shown in Figure 3.

Table 5. Effect of thiamin levels on HCN concentration of ten sorghum genotypes at the third cutting.

genotypes	Thiamin concentration mg.L^{-1}			Mean
	0	75	150	
V1	153.3	137.3	106.3	132.3
V2	176.7	164.3	167.7	169.6
V3	198.0	88.3	152.3	146.2
V4	181.7	93.0	180.3	151.7
V5	177.0	115.7	219.3	170.7
V6	186.7	127.0	211.0	174.9
V7	205.7	128.7	223.0	185.8
V8	182.7	169.0	90.7	174.4
V9	162.3	78.3	120.3	120.3
V10	126.0	95.0	134.3	118.4
Mean	175.0	119.7	160.5	27.52
L.S.D		/5.17		
		46.45		



CONCLUSION

The results of this study showed that all the genotypes which used in this study had content of the HCN in safe concentrations, when cuttingat 50% flowering in three cuttings therefore are suitable for animals feeding, in addition, use of thiamine (vitamin B1) with a concentration 75 $mg.L^{-1}$led to reduce the plants content ofHCN, therefore, thiamin (vitamin B2) can be used as an alternative to fertilizers in improving vegetative growth and forage quality.

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رش الثيامين وأثره في محتوى) HCN حامض الهيدروسيانيك (في) عشرة اصناف من الذرة البيضاء (*Sorghum bicolor* L.Moench)

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نفذت تجربة حقلية في حقول قسم المحاصيل الحقلية كلية الزراعة جامعة الانبار (ابو غريب الموقع البديل) خلال الموسم الصيفي 2016في تربة مزيجية طينية غرينية. لدراسة تأثير تداخلالاصناف مع الرش بفيتامين B2 في محتوى النبات من حامض الهيدروسيانيك (HCN). نفذت تجربةعاملية بتصميم القطاعات العشوائية الكاملة وبثلاثة مكررات ,حيث كان العامل الاول عشرة أصناف من الذرة البيضاء (أنقاذ و رابح و كافيير و ومبروك و بحوث 70 و سوري و بابل و الوركاء و ليلو و عشتار) والعامل الثاني ثلاثة تراكيز من فيتامين B2 (الثيامين) . أخذت ثلاث حشات خلال الدراسة عند مرحلة 50 % تزهير لمعرفة محتوى النبات من هذا الحامض، وتتلخص أهم النتائج بما يلي:كانت قراءات الحامض للعينات المأخوذة لنباتات الاصناف ضمن الحدود الامنه للحامض وللحشات الثلاث عند حش النبات في مرحلة 50 % تزهير , حيث سجل الصنف عشتار اقل متوسط للحامض بلغ 93.3 و 103.6 و 118.4 جزء بالمليون وللحشات الثلاث بالتتابع , كما ادى رش الفيتامين B2 بتركيز75 $ملغم.لتر^{-1}$ بإعطاء أدنى متوسط لمحتوى الحامض وللحشات الثلاث بلغ 99.1 و 107.8 و 119.7 $ملغم لتر^{-1}$ وكان للتداخل بين عاملي الدراسة تأثير معنوي في محتوى النبات من الحامض (HCN) وللحشات الثلاث .

كلمات مفتاحية : الذرة البيضاء ، الثيامين (فيتامين B2)، حامض الهيدروسيانيك