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EFFECT OF CUTTING AND POST-CUTTING INTERVALS ON HYDROGEN CYANIDE IN SORGHUM FORAGE GROWN UNDER RAIN-FED CONDITIONS

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Abstract

Sorghum forage either green or dry is the main source of livestock feed in dry areas of Pakistan. However, a lethal risk of hydrogen cyanide (HCN) is associated with this forage. Therefore, a study was carried out under rainfed conditions to assess HCN contents in three commonly grown sorghum cultivars viz., JS-2002, Chakwal Sorghum and Local Cultivar at three growth stages i.e. 3rd leaf (GS_1), pre-booting (GS_2) and 50% heading stage (GS_3) and at three post cutting intervals i.e., 06, 12 and 18 hours. The results of this study identified forage sorghum cultivar JS-2002 with lowest HCN content grown under rainfed conditions and is safer for livestock feeding at pre-booting stage. Moreover, this study has provided clear evidence that HCN contents were very high at early growth stages of crop in all the cultivars and decreased with the advancement of the growth stages. The post cutting intervals have also indicated that HCN contents decreased in all the cultivars as the post-cutting intervals (18 hours) were prolonged in comparison with fresh cutting. Thus, based on these results it is concluded that forage sorghum cultivar JS-2002 at GS_2 and after18 hours of cutting interval has the lowest HCN contents. The improved cultivar JS -2002 also has higher % of crude protein, crude fiber in comparison with Chakwal Sorghum and Local Cultivar. Therefore, livestock rearing farmers of rain-fed region are advised to grow JS-2002 for safer grazing of sorghum forage from HCN toxicity to animals at booting stage with more digestibility and nutritional value.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the family Poaceae is becoming an increasingly important forage crop in many regions of the world with warming climate conditions (Zerbini & Thomas, 2003). Its high resistance to drought makes it more suitable fodder crop to grow in semi-arid areas especially due to its higher productivity under dry conditions compared to corn (Tabosa *et al*., 1999). Sorghum holds promise for food, feed and fodder for human, poultry and cattle, respectively. It is, one of the most important fodder crops, largely cultivated all over the country during the summer, monsoon and winter seasons, in order to meet both green as well dry fodder requirements of the livestock. Although fodder is the cheapest form of feed for animals but the present fodder production does not meet the fodder requirement in terms of both quantity and quality (Ahmad *et al*., 2007). The major factors limiting the sorghum fodder production in number of countries are related to specific growth stages, insufficient fertilizer application and high contents of HCN (Singh *et al*., 2008). Sorghum is grown as fodder in irrigated areas while as grain as well as fodder in dry tracts of Pakistan (Malik *et al*., 2007).

There are at least 2650 species of plants including sorghum known to be cyanogenic, presence of cyanogenic glycosides, yields HCN on hydrolysis (Seigler, 1991) is poisonous to livestock (Collet, 2004). The contents of the HCN in sorghum vary depending on plant growth stage, genotype (variety) and environmental conditions i.e., drought, frost etc. Any stress that disrupts normal growth can contribute toward increased HCN toxicity. Probably the most common cause of HCN in sorghums is drought. Leaves of forage crops are the precious part of the plant which are liked by the animals due to its palatability and is affected earlier by drought than any other part of the plant (Vough & Cassel, 2000).

Growth stages of the plant as described by Vanderlip & Reeves (1972) start from stage 0-emergence to stage 9-physiological maturity. These stages were identified by developmental characteristics of the sorghum crop. The content of the Cyanogenic glycoside dhurrin in sorghum varies depending on plant age and growth conditions. There is a rapid increase in the cyanide potential of sorghum during germination and early seedling formation; thereafter it declines with plant growth stages (Busk & Moller, 2002). Besides, it is also important to note that the most digestible parts of sorghum are young green leaves and shoots which are highest in feed value. However, food value and digestibility both decrease rapidly as crop matures (Collet, 2004).

The plant produces cyanide which could be poisonous to livestock based on the concentration of HCN. For example, HCN concentration 0-25 mg/100 g dry plant tissue has been considered safe for grazing while 50-75 mg/100 g as doubtful and more than 100 mg/100 g as highly dangerous. The toxic effect of HCN has been found to be almost immediate. The effected animals show an increased rate of respiration, pulse rate, gasping, muscular twitching or nervousness, trembling, foam from the mouth, blue coloration of the lining of the mouth, spasms or convulsions and then death occurs from respiratory paralysis (Vough & Cassel, 2000).The greater the increase in drought stress, the lower the relative water content (Tingting *et al*., 2010) and increase in HCN resulted when sorghum forages got water stress conditions (Fjell *et al*., 1991). Therefore, this study was carried out to identify the suitable cultivars having low HCN content, proper growth stage and post-cutting period of the crop grown under rainfed conditions in order to provide valuable information to the farmers of livestock dependent community of warm climate.

Materials and Methods

Three cultivars of sorghum viz., JS-2002, Chakwal Sorghum and local cultivar were planted at the Pir Mehr Ali Shah-Arid Agriculture University Research Farm Chakwal Road Rawalpindi, (33° N and 42.72° E) during the summer 2008. The sorghum crop is warm season crop with summer rains and can tolerate extreme heat better than other crops. The meteorological data for rainfall and temperature recorded during the crop growth period were shown in Fig. 1.

The soil of the experimental field was loamy in texture with available phosphorous (P) 12.15 mg kg^{-1} and available potassium (K) 160 mg kg^{-1} with pH of 7.5. The history of the experimental field as per farm record was prepared after wheat harvesting with two cross cultivation followed by planking to make the seedbed firm for uniform seed germination. The seed of JS 2003 was obtained from the Fodder Research Institute, Sargodha and the seed of Chakwal sorghum was got from Barani Agricultural Research Institute, Chakwal while the seed of local sorghum cultivar was bought from the local market. The cultivars were sown in lines 30 cm apart on August 01,, 2008 in randomized complete block design with three replications. Each plot consisted of 6 m long rows and 3m apart thus having a plot size of $18m^2$. The seed @ of 75 kg ha^{-1} of each variety and fertilizer @ 60 kg ha^{-1} nitrogen in the form of urea, 35 kg ha^{-1} Phosphorus in the form of Single Super Phosphate were applied at the time of seed be preparation. The top fully expanded leave samples were taken at three growth stages designated as GS_1 i.e., 3rd leaf (10 days after germination), GS_2 i.e., pre-booting (40 days after germination) and GS_3 i.e., 50% heading (70 days after germination) from each plot. These growth stages were chosen initially based on the data of Vanderlip & Reeves (1972). Leave samples were taken from the three tested sorghum cultivars grown under rainfed conditions after 0 (fresh), 06, 12, and 18 hours of cutting at these growth stages to determine hydrogen cyanide contents using picrate method described by Bradbury *et al*., (1999) and Egan *et al*., (1998). The crude protein (percentage) and crude fiber on dry basis (percentage) were determined by using standard procedures as recommended by A.O.A.C. (Anon., 1990). The results were analyzed by using MSTATC program.

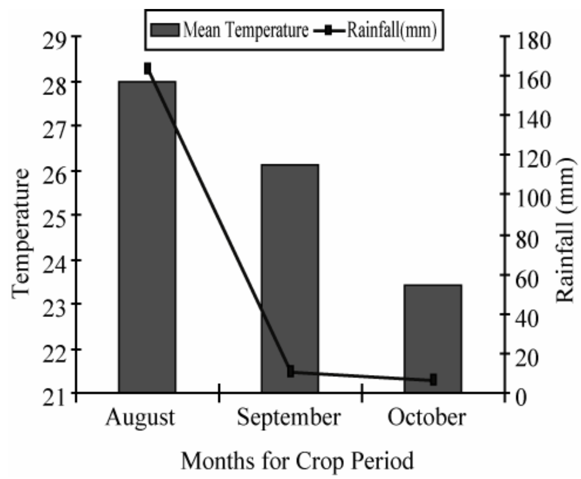

Fig. 1. The rainfall (mm) and temperature (^0C) recorded during the crop growth period.

Results

The HCN content has been described as a main contributor towards the risk related with sorghum fodder for the livestock. In this study therefore, the leaves of three different cultivars at different growth stages were used along with the post cutting intervals at 6, 12 and 18 hours including the fresh sample to determine the contents of HCN in addition to crude protein and crude fiber.

HCN contents in JS-2002 cultivar: The results of three cultivars at different growth stages indicated that there is no difference at early growth stage (GS_1) while significant difference was observed at later growth stages (GS_2 and GS_3) in all the cultivars (Table 1). Sorghum cultivar JS-2002 at GS_1 showed maximum HCN contents in fresh leaves of 71.3 ± 1.0 mg/100 g while minimum was 60.9 ± 0.9 mg/100 g after 18 hours after cutting. All the sorghum cultivars showed significant reduction in HCN toxicity at 06, 12 and 18 hours post-cutting intervals when compared with fresh cutting at early growth stage (GS_1).

Similarly, sorghum cultivar JS-2002 at GS_2 showed maximum HCN content in fresh cutting interval was 11.5 ± 1.3 mg/100 g while minimum of 8.0 ± 0.3 mg/100 g after 18 hours of cutting interval which is in permissible limit i.e., 0-25 mg/100 g. The sorghum forage cultivar JS-2002 at GS_2 showed significant decrease in HCN toxicity between fresh and 18 hours, between 06 and 18 hours and between 12 and 18 hours whereas nonsignificance difference was recorded between 6 and 12 hours of cutting intervals.

The sorghum cultivar, JS-2002 at GS_3 also showed maximum HCN content (5.6 ± 1.1 mg/100 g) in fresh leaves while minimum HCN contents of 3.3 ± 0.3 mg/100 g were recorded after 18 hours of cutting interval. This HCN contents in sorghum cultivar JS-2002 at GS_3 was significantly reduced only after 18 hours of cutting interval when compared with fresh cutting leaves however non-significant difference was recorded between 6, and 12 hours of cutting interval when compared with HCN content of fresh sampled leaves.

HCN contents in Chakwal sorghum: The Chakwal sorghum at GS_1 showed highest the HCN content of 71.4 ± 0.4 mg/100 g in fresh sampled leaves while minimum HCN content of 62.9 ± 0.2 mg/100 were recorded after 18 hours of cutting interval which is considered to be doubtful level i.e., 50-75 mg/100 g in term of safety by feeding to livestock. The HCN content in Chakwal sorghum at GS_1 showed significant reduction in HCN contents after 12 and 18 hours of cutting intervals when compared with fresh sampled leaves while no significant reduction was observed between fresh and 6 hours of cutting interval. This indicates that sorghum forage should be fed to livestock at least 12 hours after cutting for HCN safety.

Table 1. Hydrogen cyanide contents (mg / 100 g) at different cutting and post cutting intervals at different growth stages in forage sorghum cultivars grown under rain fed conditions.

Cutting interval	Fresh			06 hours			12 Hours			18 Hours		
G. stages	GS_1	GS_2	GS_3	GS_1	GS_2	GS_3	GS_1	GS_2	GS_3	GS_1	GS_2	GS_3
Cultivars↓ JS-2002	71.3a ± 1.0	11.5mn ± 1.3	5.6q ± 1.1	68.5b ± 3.0	10.5no ± 0.5	4.6qr ± 0.9	63.8de ± 1.6	10.4no ± 0.7	4.2qr ± 0.1	60.9f ± 0.9	8.0p ± 0.3	3.3r ± 0.3
Chakwal sorghum	71.4a ± 0.4	17.4j ± 0.7	11.0n ± 0.5	71.3a ± 0.9	15.8k ± 0.7	9.7op ± 0.5	64.8cd ± 0.2	15.0k ± 0.0	8.8p ± 0.3	62.9e ± 0.2	13.3l ± 0.3	8.0p ± 0.2
Local sorghum	72.3a ± 2.4	20.9g ± 0.4	12.6 ± 0.7	72.3a ± 0.3	19.9gh ± 0.6	11.8lm ± 0.6	65.8c ± 0.9	18.9hi ± 0.7	11.2mn ± 0.5	63.7de ± 1.5	17.6ij ± 0.5	10.4no ± 0.5

Treatments = 3x3x4

GS_1 = 3rd leaf growth stage, GS_2 = Pre-booting stage, GS_3 = 50% heading stage

Standard Error for Comparison (12 D. F.) = 0.7672, R Square = 0.99, Coefficient of variance (C.V.) = 3.11,

Same letters represent non-significance while different letters represent significance at ($p \leq 0.05$)

Similarly, maximum HCN content in Chakwal sorghum was found in fresh cutting leaves at GS_2 with value of 17.4 ± 0.7 mg/100 g while minimum 13.3 ± 0.3 mg/100 g after 18 hours of cutting interval. At this growth stage, significant decrease in HCN contents in Chakwal sorghum cultivar was recorded in all post cutting intervals when compared with HCN content of fresh sampled leaves. Significant reduction was noted between 6 & 18 hours and 12 & 18 hours while nonsignificant difference was noted between 6 & 12 hours interval after cutting.

At GS_3, the maximum HCN content in Chakwal sorghum was found again in fresh sampled leaves of 11.0 ± 0.5 mg/100 g while after 18 hours of cutting interval minimum HCN contents of 8.0 ± 0.2 mg/100 g were recorded. At this stage, the HCN contents in Chakwal sorghum showed significant reduction in HCN content after 6, 12, and 18 hours intervals when compared with fresh sampled leaves and also between 6 and 18 hours of cutting intervals while non significant difference was recorded in adjacent intervals such as between 6 and 12 hours and between 12 and 18 hours of cutting intervals.

HCN contents in local cultivar: At GS_1, the local sorghum cultivar, maximum HCN contents of 72.3 ± 2.4 mg/100 g was recorded in fresh sampled leaves while minimum HCN content of 63.7 ± 1.5 mg/100 g was recorded after 18 hours of cutting interval. At this stage, significant reduction in HCN content of local sorghum cultivar was recorded after 12 and 18 hours when compared with fresh cutting leaves. Similarly, significant difference was observed in HCN content recorded after 12 and 18 hours of intervals in comparison with 06 hours of cutting interval and also between 12 and 18 hours of cutting intervals while nonsignificant difference was obtained between fresh and 6 hours of cutting interval.

The maximum HCN content of 20.9 ± 0.4 mg/100 g was determined at GS_2in local cultivar at fresh cutting while minimum of 17.6 ± 0.5 mg/100 g after 18 hours of cutting interval. The significant low level of HCN content in local cultivar at GS_2 was present after 12 and 18 hours in comparison with fresh cutting. Also statistically significant difference was recorded between 06 and 18 hours intervals after cutting while nonsignificant difference was recorded between 6 and12 hours and also between 12 and 18 hours of cutting interval.

At GS_3, the maximum HCN contents of 12.6 ± 0.7 mg/100 g were recorded in local sorghum cultivar while minimum HCN content of 10.4 ± 0.5 mg/100 g after 18 hours of cutting interval were found. This showed that at GS_3, the HCN content in local sorghum cultivar significantly reduced only after 18 hours of cutting when compared with fresh sampled leaves and also between 6 and 18 hours of cutting interval whereas non significant reduction was observed in adjacent intervals such as between 6 and 12 hours and 12 and 18 hours of cutting intervals.

Crude protein and HCN content: The results of crude protein content showed the highest percentage in all cultivars at GS_2 followed by GS_3 whereas the lowest in GS_1. JS-2002 cultivar found to have highest percentage of protein at GS_2 in comparison to Chakwal sorghum and local cultivar (Table 2). The results of crude fiber indicated the highest percentage of crude fiber at GS_3 in all cultivars followed by GS_2 and GS_1.

This showed that crude fiber increased with the passage of time and with the advancement of crop growth stage. It is also observed that there is no significant difference in crude fiber content at all three stages between JS-2002 and Chakwal sorghum while significant reduction was noted in local sorghum cultivar when compared with JS-2002 and Chakwal sorghum. This also indicates that crude protein has opposite trend to crude fiber as crude protein decreased after GS_2 while crude fiber percentage increased with the advancement of crop growth (Table 2). The relationship results between HCN (percentage) and crude protein (percentage) are shown in Fig. 2. The results describe that HCN content was very high at GS_1 and rapidly decreased in GS2 and GS3 whereas, crude protein content was very low at GS1 and maximum at GS2. R-square value indicated the relationship between HCN and crude protein which is for JS-2002, Chakwal sorghum and local cultivar was 0.825, 0.842 and 0.864 respectively.

Table 2. Crude protein (percentage) and crude fiber (percentage) of three sorghum cultivars at different growth stages.

G. Stages	Crude protein (%)			Crude fiber (%)		
	GS_1	GS_2	GS_3	GS_1	GS_2	GS_3
Cultivars↓ JS-2002	3.5f ± 0.2	9.3a ± 0.2	7.4c ± 0.2	18.3d ± 1.52	30.7b ± 2.51	38.7a ± 2.0
Chakwal sorghum	2.9fg ± 0.3	8.5b ± 0.1	6.9d ± 0.1	16.3d ± 2.5	31.7b ± 3.0	38.0a ± 0.5
Local sorghum	2.7g ± 0.3	6.3d ± 0.3	5.2e ± 0.1	12.7e ± 1.5	25.7c ± 1.5	31.7b ± 2.0
C.V.	**5.16**			**7.62**		

Treatments = 03x03
GS_1 = 3rd leaf growth stage, GS_2 = Pre-booting stage, GS_3 = 50% heading stage
Coefficient of variance (C.V.), R Square = 0.99,
Same letters represent non-significance while different letters represent significance at ($p \leq 0.05$)

The relationship between HCN content (percentage) and crude fiber (percentage) is given in Fig. 3. The results indicated that HCN content decreased whereas crude fiber was increased with the advancement of growth stages. The relationship shown by R-square value was 0.902 for JS-2002, 0.964 for Chakwal sorghum and 0.966 for local cultivar.

Rainfall and HCN content relationship is depicted in Fig. 4. The results showed that HCN content decreased with decrease in rainfall amount in three different months i.e., August, September and October. The R-square value for JS-2002, Chakwal sorghum and local cultivar was 0.816, 0.828 and 0.852 respectively. Similarly, HCN content and temperature relationship is depicted in Fig. 5 which showed that HCN content decreased with decreased in temperature in three different months i.e., August, September and October with R-square value for JS-2002, Chakwal sorghum and local cultivar 0.7335, 0.747 and 0.7744, respectively.

Discussions

The results of this study showed that the level of HCN content progressively decreased significantly in all cultivars with the advancement of growth stages of the crop. Amongest cultivars, JS-2002 was found to have the lowest HCN content at GS_2 and GS_3 when however, HCN was almost similar in all cultivars at GS_1. This indicates that the HCN content of sorghum crop may be significantly influenced by genetic makeup of the cultivars. The role of genetic makeup has been pointed in the study carried out by Singh *et al*., (2008) in which three different fodder sorghum genotypes HC-308, CSV 15 and SU-1080 were investigated for HCN. It was observed that SU-1080 had significantly higher plant height at various stages of crop growth, green as well as dry fodder yield and minimum HCN content at 20, 40, 60 days after sowing and at harvest in comparison to CSV-15 and close to HC-308. These results are in accordance to Bush & Moller (2002) who observed maximum HCN content at early growth stage, which could be attributed to the high activity of enzymes (CYP79 A1 and CYP79 E1) or their biosynthesis involved in the production of HCN. The enzyme activities may have then decreased with the advancement of the crop growth stages as also noted in this study by determining the content of HCN. In another study carried out by Haque & Bradbury (2001), it was noted that total cyanide contents of sorghum leaves were 74 mg/100 g one week after germination but reduced to 16 mg/100 g three weeks later. Similar observation of drastic decline in HCN contents as the plant aged (growth stages) was made by Wheeler *et al.*, (1990).

As far as the effect of post cutting intervals on HCN is concerned; the results showed a significant reduction in HCN content after 18 hours of cutting interval in all the three cultivars tested at three growth stages. However, the study of Takamitsu (1973) on hybrid sorghum (Pioneer-988) showed that the content of HCN decreased after keeping the plant material under shade for 06 hours. This difference may be due to different group of sorghum used in the investigations.

Wheeler *et al.*, (1984) and Vickery *et al.,* (1987) reported that HCN content was reduced due to higher temperature and contradicts with the findings of Wheeler *et al*., (1990) who could not found any response of temperature on HCN content, whereas in our study HCN followed a closely negative relationship with rainfall and positive relationship with temperature as shown in Fig. 4 and Fig. 5. In general, all the cultivars showed curvilinear relationship with rainfall and temperature. The sorghum cultivar JS-2002 clearly indicated more response of temperature as well as rainfall than the local sorghum cultivar. The Chakwal sorghum cultivar behaved in between the local sorghum and JS 2002. This effect of rainfall and temperature is intermingled with growth stage as the temperature and rainfall is the mean monthly values recorded during the crop growth period. A separate study of HCN with moisture and temperature can more clarify the effects of both the climatic variables.

The highest percentage of crude protein was recorded in sorghum cultivar JS-2002 at GS_2 and decreased as the crop growth moved toward maturity while the percentage of crude fiber increased with the advancement of crop growth from GS_1 to, GS_2, and to further GS_3 in all three cultivars. These results are in line with the findings of Asharf *et al.,* (1995) and Collet (2004), who reported decrease in crude protein (%) from the vegetative stage to maturity while increase in crude fiber with the advancement of growth stage. The relationship between HCN percentage and crude protein percentage showed negative relationship that HCN content was very high at early stage i.e., (GS_1) whereas protein contents were very low in all three cultivars. However, with the advancement of the growth stages, HCN contents were decreased and crude protein increased till GS_2 and then again decreased at GS_3 as shown in Fig. 2. HCN content percentage and crude fiber percentage relationship was again strongly negative as shown in the Fig. 3. The relationships between them indicate that HCN contents were decreased as the growth stages increased whereas crude fiber percentage increased with the advancement of the growth stages.

The study has provided clear evidence that sorghum forage cultivar JS-2002 is the best in comparison with Chakwal and local sorghum cultivars grown under rainfed conditions as it has low HCN content. Further, GS_2 i.e., pre-booting and 18 hours of post-cutting interval is safe from HCN toxicity to use it as forage for livestock.

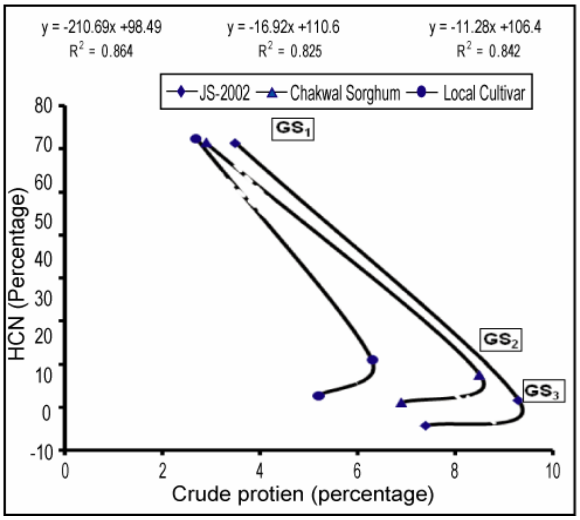

Fig. 2. Relationship between the HCN contents (percentage) and crude protein (percentage) at different growth stages in different cultivars.

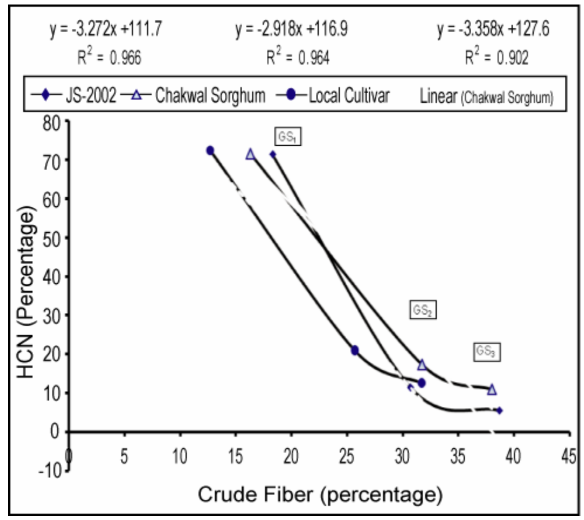

Fig. 3. Relationship between the HCN contents (percentage) and crude fiber (percentage) at different growth stages in different cultivars.

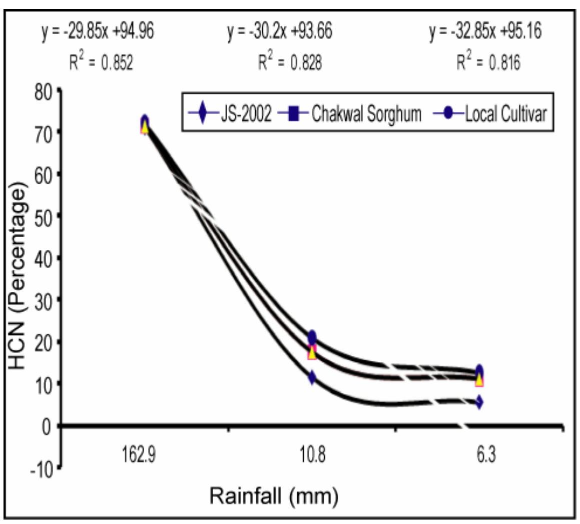

Fig. 4. Relationship between HCN contents (percentage) and rainfall (mm) in three cultivars.

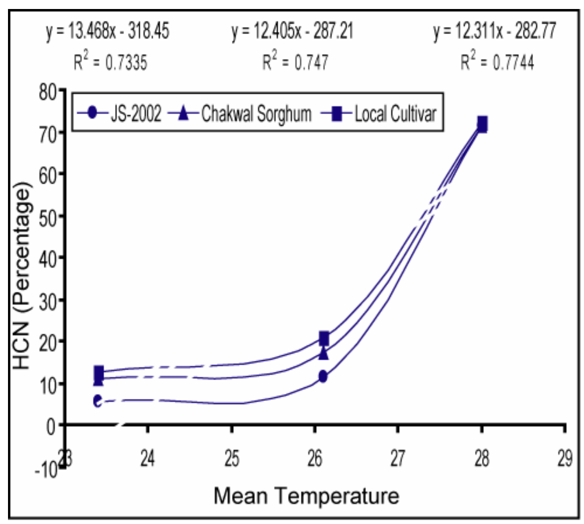

Fig. 5. Relationship between HCN contents (percentage) and temperature (centigrade) in three cultivars.

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