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An Analysis of the Hydrogen Cyanide Concentration in Four Cassava Varieties Grown in Lukenya University Farm, Makueni County, Kenya

By

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Abstract
Cassava (*Manihot esculent*) is a cyanogenic plant which can be toxic when consumed without sufficient processing. This study was carried out to determine the levels of hydrogen cyanide in different cassava varieties namely *Mzungu, Kasukari, Kitwa* and *Makueni local* grown in the South Eastern parts of Kenya at the Lukenya University farm. It also aimed at analysing both the phenotypic traits of the four cassava varieties. The experiments were carried out in a completely randomized block design with four replicates, under open field. Results obtained were subjected to the analysis of variance (ANOVA) and the means were separated using Least Significant Difference and the level of significant difference determined at 95%. Pearson's correlation analysis was performed to assess the relationships between the measured parameters. The results showed that the colour of the petiole was different for each variety that ranged from purple (*Kasukari*), cream (*Kitwa*), dark brown (*Makueni local*) to red (*Mzungu*. Significant differences were detected in the number of leaflets ($p<0.001$), plant height ($p<0.001$) and number of tubers ($p<0.001$) from the different varieties. Further, results showed that there were significant differences in the hydrogen cyanide (HCN) concentration between varieties and the different parts of the plant ($p<0.001$). It is noteworthy that all four types of cassava had cyanide levels that were higher than the HCN World Health Organization (WHO) recommended limit (10mg/kg body weight). The Pearson's correlation analysis showed that there was a significant positive relationship between the number of leaflets and the plant height ($r^2 = 0.590$) and the number of tubers per plant ($r^2=0.562$). The findings in this study indicate that the cassava varieties have significantly high levels of cyanide thus, a sensitization campaign is advised to educate consumers on the processing techniques prior to consumption. It also calls for the implementation of criteria for acceptable levels of HCN in cassava food items that are globally recognized. The phenotypic characterization can be used by farmers on choice of variety and breeding purposes to improve cassava yields in this region and beyond.

Key Words: Toxic, Breeding, Phenotype, Lukenya University, Makueni County

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Introduction

Cassava (*Manihot esculenta*) is a tropical American perennial crop. In the 16th century, Portuguese traders brought it from Brazil to Africa (Samura & Fomba, 2019). The plant parts that are used include the storage root (tuber) and leaves (Moreno-Cadena et al., 2021). It belongs to the dicotyledon family Euphorbiaceae. The *Manihot* genus contains approximately 100 species, with *Manihot esculenta* being the only one that is commercially produced. There are two different forms of cassava plants: spreading and erect, which may or may not have branches at the top (Samura & Fomba, 2019). According to a study done by (Fernandez et al., 2017), Millions of people in the tropical parts of Africa, Latin America, and Asia depend on cassava, a widely grown tuber crop. Cassava is a widely-liked crop since it is simple to grow and produces well in both excellent and bad soil conditions, (Ogbonnaya 2018). It thrives on soils ranging in texture from sands to clays and on soils of relatively low fertility. It grows best on light sandy loams or on loamy sands that are moist, fertile, and deep (Marigi et al., 2016).

In reality, cassava can grow on a wide variety of soils as long as they are friable enough to support the development of the tubers. The plant may grow stems and leaves instead of tubers when the soil is highly rich in the elements essential for basic plant nutrition, such as phosphorus, nitrogen, and potassium. The ideal regions for cassava growth, according to Halewood (2011), are those with a mean temperature of 25 to 29 C and a soil temperature of around 30 C. In environments with temperatures below 10 C, the plant cannot live. Additionally, the plant thrives in environments devoid of frost throughout the year. Since cassava is a tropical crop, its short days prevent starch from being stored, which results in low yields. In areas lower than 1500 meters above sea level, cassava is widely grown (Acland, 1985). Although the plant's starchy root is extensively consumed globally, many regions also eat its fresh leaves. Because it thrives in poor soil without fertilizer and can withstand drought, cassava is gaining popularity as a food source (El-Sharkawy, 2004). For a three-year food reserve, roots can be left in the ground. When there is a drought, the leaves fall off, but the plant is kept alive by its deep roots; when it rains, the leaves grow back (Bradbury and Holloway, 1988).

With 118 million metric tonnes produced throughout the continent in 2010, cassava had the largest overall production in Africa. It also provided a significant amount of energy to the population, averaging 196 kcal per person per day in 2008. (FAO, 2010). Over 500 million people, many of whom are poor, live on it as a primary food in the tropics (Halewood, 2011). Due to the crop's variable harvesting schedule, growers are able to keep the roots in the ground until they are required (Iglesias et al., 1997). In addition, the crop performs admirably under situations that restrict growth. Due to its resistance to drought and barren soils, as well as its ability to recover from disease and pests, it is regarded as a famine reserve crop in Africa.

In 2002, it was estimated that 184 million tonnes of cassava roots would be produced. By 2008, that number was expected to rise to 230 million tonnes. (Frederick et al., 2008) In

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2002, Latin America and the Caribbean produced 33.2 million tonnes, followed by Asia with 51.5 million tonnes (FAO, 2005). The biggest cassava producer in the world is Nigeria. 77% of all dried cassava exports worldwide in 2005 came from Thailand, the world's top exporter. (Katz and Weaver, 2003). With 13.6% of global exports, Vietnam ranks second, behind Indonesia (5.8%) and Costa Rica (2.1%). (FAO, 2005). Kenya's western, eastern, central, and coastal regions are where cassava is farmed. In terms of importance, it is second only to maize in Kenya's western and coastal regions. FAO forecasted in 1986 that Kenya had 30,000 ha of cassava agriculture, generating 380,000 tons (Nweke, 1996).

A large amount of variation exists among the cassava leaf stem and root characteristics. These traits which include leaf morphology, stem colour, branching habit and storage root, shape and colour may influence cassava yield (Dixon, 2019). Therefore, a proper understanding of these variations in plant characteristics assists in the selection of cassava types with the desired traits which in turn will contribute to improved crop establishment and increased yields.

In Kenya, cassava is grown in a number of locations and has both beneficial and harmful impacts on people. On the one hand, the plant's roots and leaves are nutritious since they include carbohydrates, proteins, vitamins, calcium, and iron, but on the other, they do contain cyanogenic glycosides. A molecule called hydrogen cyanide is produced when cyanogenic glycosides are digested by the enzyme linamarase. This chemical can result in severe poisoning and even death, particularly if levels exceed the WHO-recommended minimal limit of 10 mg HCN equivalent/kg body weight. Studies on cyanide concentration in cassava have been done, but none has been done on cassava from Kenyan markets.

Despite efforts to create high-yielding cultivars, farmers have had a difficult time embracing these new varieties. However, fresh reports of cassava root tuber bitterness pose a danger to the adoption rate of improved cassava cultivars. It is necessary to locate and treat the sources of the bitter flavor in high-yielding improved cassava varieties. Although the improved cultivars expand more quickly and have greater pest and disease resistance, hydrocyanic acid has been associated with poorer cassava root quality. This is the main justification for the rejection of cassava cultivars in Eastern Kenya and other "boil and eat" cultures like the coastal area (Sayre, 2004). In addition, few studies have been previously done in the study region to look at the phenotypic characterization and hydrogen cyanide concentration of cassava varieties grown.

Statement of the Problem

A large amount of variation exists among the cassava leaf stem and root characteristics. These traits which include leaf morphology, stem colour, branching habit and storage root, shape and colour may influence cassava yield (Dixon, 2019). Therefore, a proper understanding of these variations in plant characteristics assists in the selection of cassava types with the desired traits which in turn will contribute to improved crop establishment and increased yields.

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Review of Related Literature

The term "cyanide" refers to all cyanide compounds whose cyanide ion (CN) may be identified (Adamolekun, 2012).A substance with carbon in it is referred to as a biochemical component and is present in all living things. The total amount of cyanide present as HCN or CN- is known as free cyanide (Haque and Bradbury, 2002). Thousands of different species of bacteria, algae, fungus, plants, and insects produce, excrete, and decompose cyanide and chemically similar chemicals in nature. Low quantities of cyanide can therefore exist in naturally occurring surfaces like groundwater, where they would ordinarily not be anticipated to (Anning, et al., 2019).

Three groups of cyanide compounds are normally recognized. The first category, referred to as free cyanide, is made up of the cyanide ion CN- (produced when sodium or potassium cyanide dissolves in water), as well as the hydrogen cyanide gas (HCN). The second category is made up of weak to moderately strong complexes between the cyanide ion and some metals, such as Zn, Ni, Ag, Cd, and Hg. The third category is made up of strong complexes between the cyanide (Anning, et al., 2019).

Cigarette smoke and smoke produced when some synthetic materials, such plastics, are burned, are the two main sources of cyanide in the air. Upon metabolism, the blood pressure-lowering medication sodium nitroprusside and the anti-cancer treatment laetrile both emit cyanide (Haque and Bradbury, 2002).

There are at least 2650 species of plants that contain cyanoglycosides and generally with a corresponding hydrolytic enzyme (β-glycosidase). One or more glycosidases and -hydroxynitrile lyases catalyze the hydrolysis of cyanogenic glycosides, which is the primary mechanism by which cyanogenesis occurs in plants. The plant's subcellular or tissue level contains these substrates and enzymes in distinct, intact vacuoles, preventing autotoxicity and hydrolysis under physiologically normal conditions (Vetter, 2000).

Nevertheless, a predator's disruption of the tissue enables the mixing of the substrate and the enzymes that start the cyanogenesis and subsequent hydrolysis (Castada et al. 2020).This then decomposes into a sugar and a cyanohydrin, which quickly breaks down into hydrogen cyanide and an aldehyde or ketone (Haque and Bradbury, 2002). When the seeds are crushed and moistened, the enzyme emulsin causes this reaction in the kernels (Haque and Bradbury, 2002). Amygdalin is transformed into glucose, benzaldehyde, and hydrogen cyanide (and is also found in peach stones and bitter almonds) (Chiwona-Karltun et al., 2004). The principal cyanogenic glycosides are present in the edible sections of plants like

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almonds, sorghum, cassava, stone fruit, and bamboo shoots. There are about twenty-five identified cyanogenic glycosides.

Many different types of microorganisms have been found to be able to change cyanide compounds, whether they are simple or complicated. These microorganisms include algae like Scenedesmus obliquus, bacteria like Klebsiella oxytoca (Castada et al. 2020) ,fungus like Fusarium oxysporum (Akinpelu et al., 2015. Cyanide is used as a nutrient by bacteria during the cyanide breakdown process by microorganisms, acting as a source of nitrogen. Cyanide compounds can be used by some bacteria as a source of both carbon and nitrogen. In this situation, the microbes no longer require an external source of carbon and nitrogen.

Numerous foods and plants contain cyanides, which are created by specific bacteria, fungus, and algae. In order to protect plants from herbivores, cyanides are typically linked to sugar molecules in the form of cyanogenic glycosides (Vetter, 2000).

Methodology

Study Area

The project was carried out at Lukenya University farm, Ngwata, Makueni County. It is located within the coordinates 2.2559° S, 37.8937° E. The area has well-drained and light-textured, sandy loam and fertile soils. It experiences a bi-modal rainfall pattern with long rains received between March and May and short rains between October and December with June-September as dry months with average rainfall for each season ranging from 150-650mm with mean minimum and maximum annual temperatures range of 14.3℃ and 35.1℃, respectively

Experimental Materials and Design

The propagating materials (stem Cuttings) were sourced from the Makueni farmers. Cultivars of four varieties namely *Kasukari*, *Mzungu*, *Kitwa and Makueni Local* were established in an open field in single rows in a Randomized Complete Block Design (RCBD) with four replicates on ridges 1.0 M apart, with a 1.0M spacing between plants within the rows. Each variety was planted in a plot size of 10M by 10M. Mature Stem cuttings of about 30cm long containing between 10-12 nodes were planted in a vertical position along the top of ridges at an angle of 45˚. Irrigation was applied for the first three months to get even growth. Throughout the experimental period, earthing up, weeding, and supplementary irrigation activities were carried out uniformly whenever required.

Phenotypic Analysis

The plants were randomly sampled to allow equal chances for all the plants to be selected for study to provide a completely representative sample (Singh, 2003). 30 plants per variety were randomly selected, observed and recorded for their phenotypic characteristics.

Hydrogen Cyanide

Six months after planting, the first fully expanded leaf from the top of a cassava plant, two leaves below it and a piece of the root picked and put in well-labelled paper bags. They were then taken to laboratory for further processing according to (Bradbury, 1999).

Laboratory Procedures

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A UV-visible spectrometer, Cecil CE 2041 2000 series (Cambridge, England, United Kingdom) was used for analysis and the electronic analytical balance was used for weighing the samples. Analytical reagents such as Ninydrin, Potassium cyanide, Sodium chloride, Sodium carbonate, Sodium hydroxide and sodium hydrogen carbonate were used. A calibration graph was first constructed using standard solutions of CN^- at concentrations of 0.02,0.04,0.08,0.1 and 0.2 µg/mL (which is within the linear range) and was prepared by adding appropriate volumes of cyanide solutions at concentration of 20 µg CN^-/mL to 1 mL of 2% Na_2CO_3

Processing cassava tubers and leaves

The cassava root tubers were properly washed to remove the soil. The dark brown scale covering (periderm) was removed using a clean stainless knife and the leaves cleaned using distilled water, chopped into small pieces. 10.0g of the weighed samples were air dried at room temperature.

Determination Of Cyanide In Cassava Tubers And Leaves

To each standard cyanide solution, ninhydrin solution (0.5 mL) containing 5 mg/mL of 2% NaOH was added. For the purpose of color development, the mixture was homogenized and incubated for 15 minutes. The blank was made similarly, with the exception that 1 mL of 2% $Na2CO_3$ lacking CN was added in place of the usual 1 mL. Using a UV/Vis Spectrophotometer at 485 nm, the reaction product (Cyanide-ninhydrin adduct) of the various doses of cyanide was evaluated for UV-Visible absorption. By adding 10 g of the ground sample to a standard volumetric flask (5 mL) and bringing the volume to the proper level with 0.1% $NaHCO_3$, the total cyanide in the samples was calculated. The samples were sonicated for 20 minutes in a water bath and the mixture centrifuged at 10,000 rpm for 10 minutes. The supernatant was pipetted using an automatic pipette, two aliquots (2 mL each) and added to 0.5 mL ninhydrin in NaOH. This was allowed to sit for fifteen minutes for colour development and absorbance measured at 485 nm.

Data Analysis

Results obtained were subjected to the analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.4. The means were separated using least significant difference (LSD) and the level of significant difference determined at $p < 0.05$.

Results

Characteristics observed of four cassava varieties

The four cassava varieties were distinguished based on the morphological characteristics of the petioles, stems and roots. (Table 1). The stem/internode colour was greyish green for the *Kitwa* and *Kasukari* varieties and brownish green for the *Makueni local* and *Mzungu* varieties. For the root pulp, all the varieties were cream in colour except for the *Mzungu* variety that was brown (Table 1).

Table 1: Observed phenotypic characteristics of the cassava varieties

Plant trait	Variety			
	Kasukari	*Kitwa*	*Makueni local*	*Mzungu*
Colour of petiole	Purple	Cream	Dark brown	Red

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Stem and internode colour	Greyish green	Greyish green	Brownish green	Brownish green
Colour of root pulp	Cream	Cream	Cream	Brown

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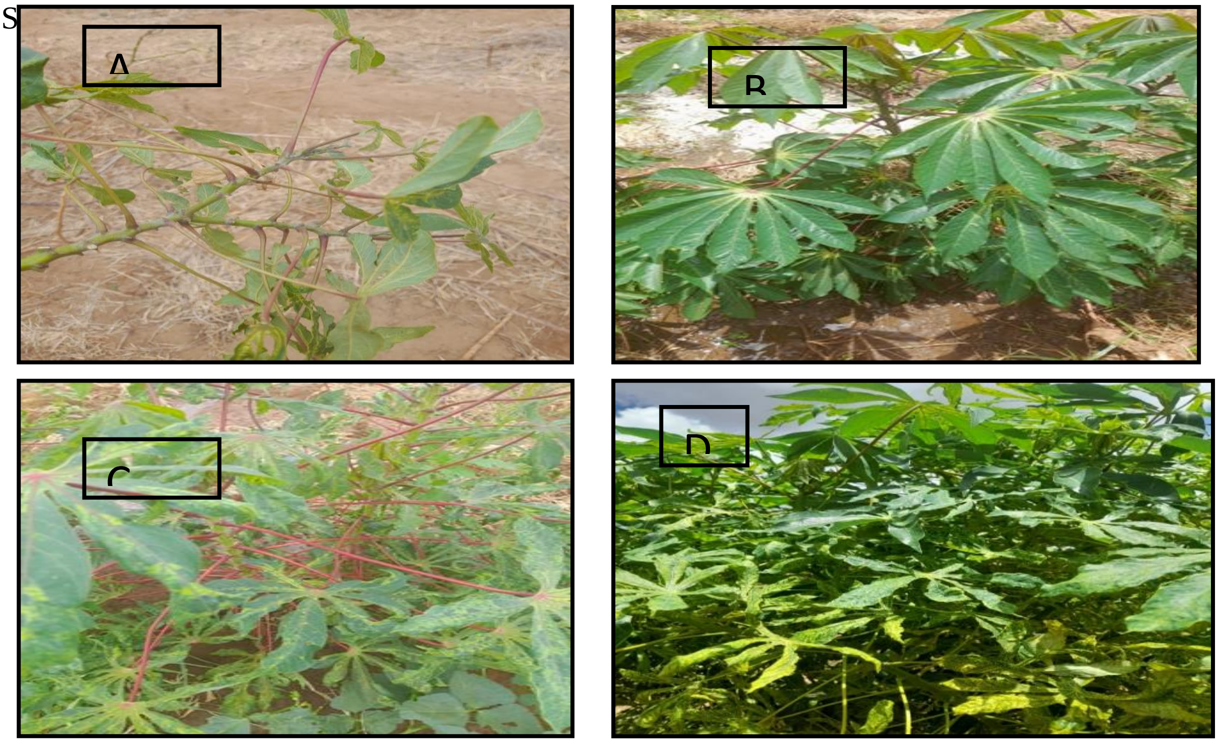

Photo 1: Observable phenotypic traits of the cassava varieties. Makueni local (A), Kasukari (B), Mzungu (C) and Kitwa (D).
Source: Field Data 2022

Effect of cassava variety and plant part on the hydrogen cyanide (HCN) concentration
Results showed significant differences in the hydrogen cyanide concentration among the four cassava varieties (p<0.001). Similarly, there were significant differences in HCN concentration between the different parts of the plant (p<0.001) (Table 4.3). A significant variety and plant part interaction was also observed (p<0.001) (Table 4.3).

Table 2: Analysis of variance mean squares values for the HCN concentration in cassava

Source of variation	df	HCN
Replicate	3	84.84
Variety	3	621.44^{***}
Plant part	1	2101.46^{***}
Variety*Plant part	3	302.01^{**}
Error	21	58.45
Total	31	
Minimum		44.38
Maximum		90.68

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CV (%)	11.1
R^2	0.81

, * - significant correlation at p= 0.01 and p= 0.001 levels of significance respectively.
Source: Field Data 2022

The *Kitwa* variety recorded the highest hydrogen cyanide concentration (78.4 mg/kg) compared to the other varieties (Figure 1). However, this was not significantly different from the *Makueni local* variety. The *Kasukari* and *Mzungu* varieties had significantly lower hydrogen cyanide concentration compared to the *Makueni local* and *Kitwa* varieties (Figure 4.4).
In terms of the plant part effect, the leaves recorded significantly higher hydrogen cyanide concentration (76.9 mg/kg) compared to the roots that recorded 60.7 mg/kg HCN concentration (Figure 2).

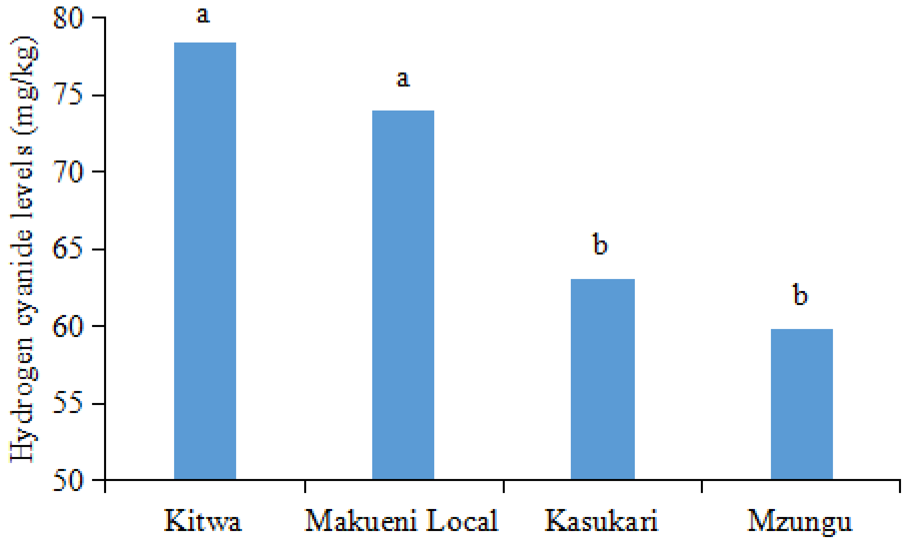

Figure 1: Effect of variety on the hydrogen cyanide level concentration. Values are the average HCN concentration for both the leaves and roots.
Source: Field Data 2022

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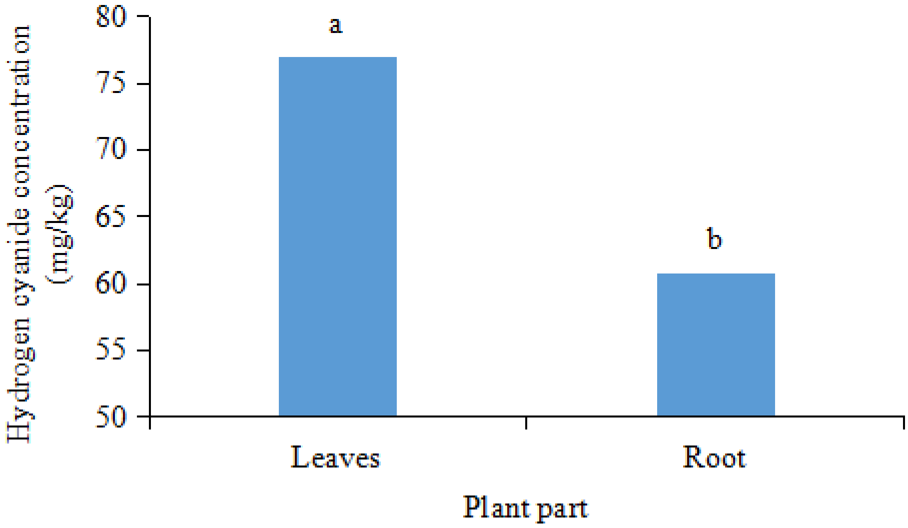

Figure 2: Effect of plant part on the hydrogen cyanide concentration in cassava. Values are the average HCN concentration for both the leaves and roots.
Source: Field Data 2022

In terms of the differences in the leaves and root HCN concentration, there were no significant differences among the four cassava varieties for the leaves HCN concentration (Table 3). However, there were significant difference in the root HCN concentration among the cassava varieties. *Kitwa* and *Makueni local* varieties had significantly higher HCN concentration compared to the *Kasukari* and *Mzungu* varieties. The *Kasukari* variety recorded the least HCN concentration in the roots (Table 3; Figure 4.6).

Table 3: The hydrogen cyanide concentration in the leaves and roots of cassava varieties

Variety	Leaves	Roots
Kitwa	80.3a	76.4a
Kasukari	79.2a	46.9b
Makueni local	79.0a	69.1a
Mzungu	69.1a	50.5b
LSD ($\alpha \leq 0.05$)	NS	8.4

Means followed by similar letters within a column are not significantly different at $\alpha \leq 0.05$.
NS- not significant
Source: Field Data 2022

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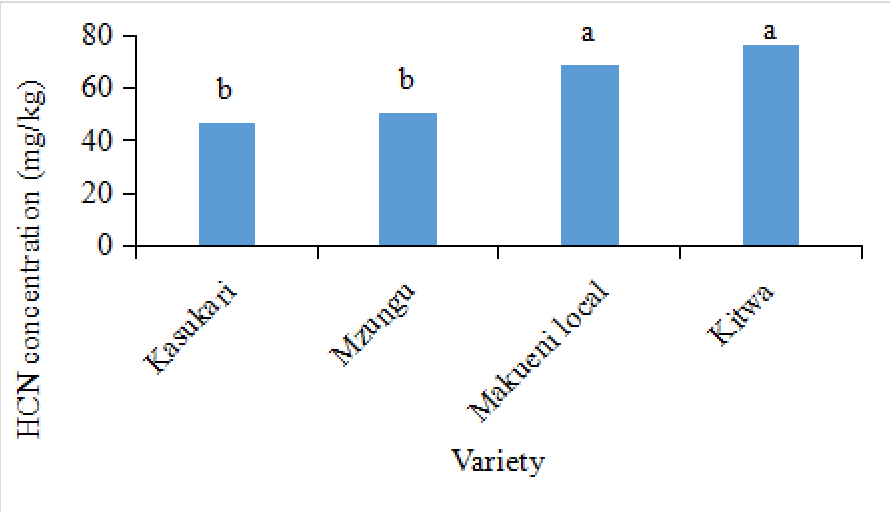

Figure 3: HCN concentration in the roots of the cassava varieties
Source: Field Data 2022

In terms of the variety*plant part interaction, there was varied observations. For the *Kasukari* variety, there was a large difference in the HCN concentration between the leaves and the roots compared to the other varieties (Figure 4). This is followed by *Mzungu* variety where a considerable difference was recorded between the HCN concentration in the leaves and roots. For the *Kitwa* variety, there was no statistical difference in the HCN concentration between the leaves and roots (Figure 5).

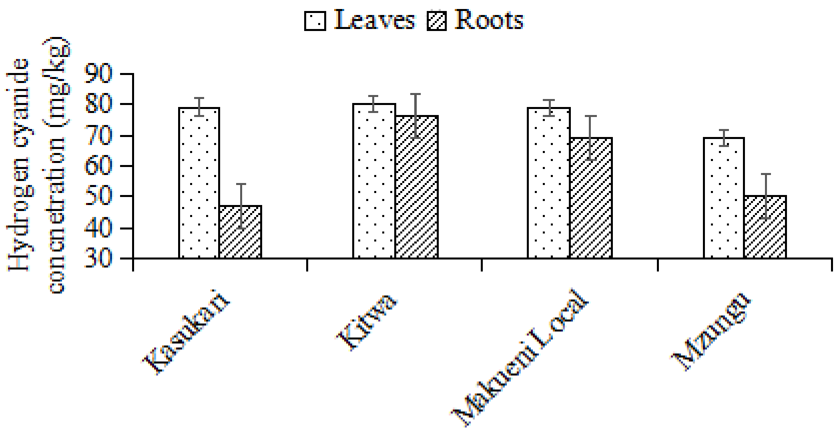

Figure 5: Interaction between the variety and plant part on the HCN concentration in cassava. Error bars represents the standard error of the means.
Source: Field Data 2022

Correlation between the phenotypic traits and hydrogen cyanide concentration

The Pearson's correlation analysis showed that there was a significant positive relationship between the number of leaflets and the plant height (r^2 = 0.590) and the number of tubers per plant (r^2=0.562). Similarly, there was a significant positive relationship between the number of tubers per plant and the plant height (Table 4). There was a positive relationship between the HCN concentration and the number of leaflets and plant height and a negative relationship with the number of tubers per plant. However, these correlations were not

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statistically different. A positive relationship was observed between the HCN concentration in the roots and in the leaves (Table 4).

Table 4: Correlation analysis among the phenotypic traits and hydrogen cyanide concentration

	No. of Leaflets	Plant Height	Number of tubers	HCN in roots	HCN in leaves
No. of Leaflets	1	0.590*	0.562*	0.172ns	0.156ns
Plant Height		1	0.670*	0.050ns	0.179ns
Number of tubers			1	-0.045ns	-0.084ns
HCN in roots				1	0.110ns
HCN in leaves					1

*, ** - significant correlation at p= 0.05 and p= 0.01 levels of significance respectively.
Source: Field Data 2022

Discussion and Conclusions

There were significant differences in HCN concentration amongst the four cassava varieties (p<0.001). Similarly, there were significant differences in HCN concentration between the roots and leaves). Cuvaca et al. (2015) explained that the cyanide level in cassava tuber appears to be a function of the physiology of the crop or possibly cassava variety. Similarly, Hue et al. (2012) reported that the HCN content in cassava was affected by the variety. Numerous previous research findings that have reported existence of large differences in HCN content between cassava varieties (Gleadow and Woodrow, 2002; Cardoso et al., 2005; Nwakaeze, 2005; Hang and Preston, 2005; Ubi et al., 2008).

The HCN concentration of the cassava varieties from this study showed that the levels were more than the recommended World health Organization (WHO) maximum acceptable level of cyanide in foods meant for human consumption which should be below 10 mg/kg (FAO, 2007). Bitter cassava varieties are associated with high concentrations of cyanogenic glucosides, linamarin and lotaustralin (Chiwona-Karltun et al., 2004) and bitter-tasting tubers in most varieties are known to have a high cyanogenic potential (Ceballos et al., 2004). Bitter cassava varieties are more drought resistant and thus more readily available and cheaper (Chijindu and Boateng, 2008). In the present study, the Kasukari variety had 46.9 mg HCN/kg in the roots. Sweet varieties of cassava have been reported to contain <50 mg/kg or between 15 to 50 mg/kg of edible portions on fresh weight basis (Food Standards Australia New Zealand, 2005) and such levels have been said to be harmless (Burns et al., 2012).

The present findings showed that the leaves recorded significantly higher hydrogen cyanide concentration (76.9 mg/kg) compared to the roots that recorded 60.7 mg/kg HCN concentration. This trend is in line with what was reported by Azucena-Topor et al. (2008) who showed higher HCN concentrations in the leaves (50.49+1.4 to 388.08+11.2) than in the roots (12.87+4.2 to 135.63+7.0). Similarly, Bellotti and Riis (2003) reported that the cassava leaves have the highest concentration of cyanogens. The concentrations of HCN differ between tissues in the same plant and even between compartments of the same tissue (Nwakaeze, 2005). The higher HCN content in the leaves was explained by Selmar (1994) who reported that leaves are expected to have higher HCN content than the roots since

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synthesis of cyanogens occurs mainly in the leaves and are translocated to the roots and other plant tissues.

Correlation of the HCN content in the roots and leaves from this study revealed a non-significant relationship. Similarly, Ubi et al. (2008) found that the total HCN content of the roots were not correlated with the content in the leaves of the same plant. Therefore, the classification of the cassava into 'sweet' or 'bitter' varieties should be based on the edible root/tuber concentration rather than the leaves' HCN content.

Recommendations

The concentrations of cyanide in cassava from all the samples were higher than the recommended level by WHO (10mg of HCN/kg body weight). The following recommendations were made from this study:

The *Kasukari* variety has lower cyanide than the other varieties studied and is recommended for farmers in the region to reduce the risk of cyanide poisoning. Public awareness campaigns should be done to sensitize the public about high levels of HCN equivalent/kg of cassava compared to the recommended standard WHO reference value Cassava consumers should be sensitized on the best and effective methods of processing cassava and encouraged to use them before consumption. Studies on cyanide content in cassava products such as flours should be undertaken. More studies should be carried out on the cyanide content equivalent/kg of protein rich cassava leaves used as vegetables

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