The cyanogenic potential of roots and leaves of released cassava (*Manihot esculenta* Crantz) varieties in Sri Lanka

N.L.A.T.S. Nanayakkara[1], G.G.C. Premalal[2], S.M.S A. Sumathipala, E.R.I.B.N.N. Jayalath[2] and N.N. Rajapaksha[1]

[1]*Horticultural Crops Research and Development Institute, Gannoruwa, Sri Lanka*

[2]*Veterinary Research Institute, Gannoruwa, Sri Lanka*

Introduction

Manihot esculenta Crantz, commonly known as cassava, belongs to the family Euphorbiaceae and genus Manihot having a great economic interest. Cassava species are classified into three groups according to their CP levels.

1) Sweet or nontoxic levels with less than 50 ppm. 2) Moderately poisonous with 50-100 ppm and 3) Bitter, toxic or very poisonous containing CP more than 100 ppm (Hidayat *et al*., 2016). For Human the lethal dosage is 1 mg/kg of live weight (Chen *et al*., 2018). Trivalent iron of cytochrome oxidizer is reacted with cyanide. Cyanide causes neurological, respiratory cardiovascular and thyroid defects, and ultimately the death. (Drochioiu *et al*., 2011). Therefore it is recommended to consume cassava with Cyanogenic potential (CP) levels less than 50 ppm. (Hidayat *et al*., 2016).

Both roots and leaves are consumed. Cassava leaves are a rich source of protein, calcium, and vitamin and used as a leafy vegetable. Highest amount of the cyanogenic potential exists in immature parts and it is safer to eat the leaves bellow the third leaf from the top (Hidayat *et al*., 2016). Many cultivars are grown all over the country in Sri Lanka, but their cyanide content is still unknown. Seven cassava varieties have been released by the Department of Agriculture. This study was initiated to determine the cyanide levels of roots and leaves of released varieties to clarify some doubtful points, vomiting and headache after consuming *Kirikawadi* have been reported occasionally. Therefore all the varieties were tested again to clarify the cyanide levels. All the released cassava varieties contain CP less than 50 ppm. Cyanide content of cassava leaves and roots varied with age, and parts of the crop, nitrogen content of the soil. Crop harvesting stage, poor weeding practices, piecemeal harvesting and branch pruning influence the cyanide levels (Imakumbili *et al*., 2019). Further studies has to be done with different agronomic traits to make sure the cyanide contents in different conditions.

***** Short Communication***

Materials and methods

The study was carried out in the experimental field of the Horticultural Crops Research and Development Institute (HORDI), Gannoruwa, Peradeniya during April to December 2016, using eight month old cassava varieties released by the DOA. Varieties were *Suranimala*, MU51, *Kirikawadi*, CARI 555, *Swarna*, *Shani* and HORDI MU 01. Crop was established before 8 months from the date of sampling and management practices were followed according to the DOA recommendations.

Chemical analysis for cyanide was done in the Pasture Division, Veterinary Research Institute (VRI) Gannoruwa, Peradeniya using Alkaline Picrate Spectrophotometric Method (Biology-assets.anu.edu.au., 2019). Roots, with and without cortex separately, and mature leaves (below third leaf) and immature top leaves (third and above) were subjected to test the HCN levels on a CRD experiment with three replicates.

The roots were cleaned and peel out the outer brown skin and cut in longitudinal direction into 1 inch sections with and without inner cortex separately. Root and leaf samples were minced using a laboratory blender. Five grams of root sample sand 1 g of leaf sample were measured and put immediately into 50 m*l* centrifuge tubes, and added 3-5 drops of chloroform, 2 m*l* of distilled water and 1 m*l* phosphate buffer solution (0.1M). All tubes were shacked well after closing the lid. Thereafter, 2 m*l* of 1% NaOH was added in to separate 5 m*l* small plastic vial and insert in to the centrifuge tube by removing the lid. Tubes were kept overnight (12 hrs) at room temperature or 2 hrs at 37 ^{o}C water bath to trap HCN in to NaOH. In the following day, cyanide reacted NaOH solution in small vial was transferred in to a another 50 m*l* Nessler tube and add 5 m*l* of picric buffer regent and 6 m*l* of distilled water and shacked well. Cyanide levels of each sample was measured with standards at 520 nm. Data were analyzed using ANOVA in SAS (Statistical Analysis Software) version 9.1 and means were compared using Duncans Multiple Range Test (DMRT).

Results and discussion

All the varieties showed cyanide levels less than 50 ppm in both leaves and roots. Roots were tested as two groups, with cortex and without cortex. Out of tested varieties significantly higher cyanide level was observed in variety *Kirikawadi* in roots without cortex. Variety *Kirikawadi*, showed the highest cyanide levels with cortex followed by *Suranimala*, Mu51, CARI555. HORDI and MU-1. The lowest cyanide level was observed in Variety CARI 555 in root flesh without cortex (16.17 ppm).

Variety *Shani* had the second lowest cyanide level (21.01 ppm) in root flesh without cortex. Significantly highest cyanide value was observed in variety *Kirikawadi* without cortex (Table 1).

Highest cyanide content was observed in immature top leaves in all varieties (3rd leaf and above) mature leaves (4th-6th) had less cyanide levels. *Kirikawadi* and CARI 555 gave the highest significant cyanide levels up to 26-30 ppm in immature top leaves. Significantly highest cyanide level 8.41 ppm was observed in variety *Kirikawadi* in mature middle leaves. All the varieties gave significantly low cyanide value than variety *Kirikawadi* in middle leaves. Variety HORDI MU-1, Shani and MU-51 gave the lowest cyanide value in middle mature leaves. All the varieties other than variety *kirikawadi* are safer to use as leafy vegetable, because the boiling time is limited in leafy vegetable preparation, poisonous cyanide may not be removed. Considering all the results in this study, variety *Kirikawadi* contains higher cyanide levels compared to other varieties, both in leaves and root.

Table 1. HCN content in Cassava Roots with and without cortex

Variety	HCN with cortex (ppm)	HCN without cortex (ppm)
Suranimala	30.58^{ab}	20.11^{b}
Mu 51	26.07^{bc}	24.91^{b}
Kirikawadi	40.08^{a}	41.82^{a}
Swarna	19.33^{bc}	19.86^{b}
CARI 555	21.61^{bc}	16.17^{b}
Shani	12.92^{c}	21.01^{b}
HORDI MU1	28.39^{ab}	23.21^{b}
CV%	27.39	28.03

Means with the same letters are not significantly different at $p< 0.05$

Table 2. HCN content in Cassava leaves

Variety	HCN in Top leaves (ppm)	HCN in Mature leaves (ppm)
Suranimala	4.04^{c}	2.20^{b}
Mu 51	4.23^{c}	1.63^{b}
Kirikawadi	30.10^{a}	8.40^{a}
Swarna	19.42^{b}	2.43^{b}
CARI 555	26.38^{a}	2.67^{b}
Shani	16.49^{b}	1.93^{b}
HORDI MU1	2.81^{c}	1.44^{b}
CV%	24.52	23.06

Means with the same letters are not significantly different at $p< 0.05$

Conclusion

In eight months old, immature cassava leaves (top bunch to 3rd leaf) contain high cyanide content while leaves from 4th leaf contain comparatively less cyanide level. Variety *Kirikawadi* gave the highest cyanide level in immature and mature leaves and roots with and without cortex. The cyanogenic potential is high in variety *Kirikawadi* both in leaves and in roots. Cyanide levels in root in other varieties was less than 30 ppm. As the cyanide content change with different agronomic traits further studies has to be made for better conclusion.

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