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PERFORMANCE OF NATURAL CHELATES IN THE ENHANCEMENT OF IODINE BIOFORTIFICATION IN CASSAVA (*Manihot esculenta* Crantz)

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ABSTRACT

The study investigated the ability of some organic chelates to enhance iodine availability, uptake and deposition in the edible root of cassava. The chelates used were EDTA as standard chelate, Bontera as commercial organic chelate and periwinkle effluents and Smoke solution as local organic chelates. The cultivars used were TME419 and TMS3168/UMUCASS/36 also known as YELLOW ROOT (YR). One hundred (100ml) of 100µg of iodine obtained from potassium iodide was added to 5000ml each of deionized water, 1ml/L Bontera, 1ml/L EDTA , Periwinkle effluents and Smoke solution. The treatments were applied through foliar application at 3 months after planting. The iodine content distribution ranged from 350µg/g (TME419 Root peel; control) to 23440µg/g (YR Stem; SS +KI). In the edible root, the bioavailable iodine ranged from 790µg/g (YR; PE +KI) to 7710µg/g (TME419; PE +KI). After processing to garri and fufu, the content ranged from 100µg/g (YR; control and PE +KI) to 1030µg/g (TME419; PE +KI) in fufu and 410µg/g (YR, PE +KI) to 4010µg/g(TME419; PE +KI) in garri. The study revealed that all the chelates had good potentials to enhance iodine biofortification. However, among the chelates used Bontera proved to be best because, it was stable when used individually and in combination with KI and was also stable in both cultivars used. Among the cultivars used YR proved to be better because, it has the ability to retain iodine for a longer period of time. The use of organic chelates should be encouraged to fast track micronutrients biofortification and availability.

Key words: Iodine, Biofortification, Cassava, Organic chelates, Bioavailability

INTRODUCTION

Trace element like iodine helps to control many of these ailments. Iodine controls blood sugar level. Iodine also controls heart beat rate, helps to maintain skin, hair and nail health. Iodine is an essential micronutrient. Iodine produces tri-iodothyronine (T_3) and thyroxin (T_4) which are essential for body hormone regulations. Iodine is needed for many functions in the human body; it gives energy, helps in growth and development.). Seafood (seaweeds and aquatic animals) are good sources of iodine. Human body does not synthesize iodine (Zelmon, 2019) hence, it all depends solely on what humans takes in as food or supplements.

The most recommended strategy to control the problem of micro nutrients malnutrition by the World Health Organization (WHO) and many researchers is biofortification (WHO, 2000; Welch and Graham, 2000; White and Broadley, 2009; Ikuli *et al*, 2017). Biofortification is the process whereby nutritional contents in food are increased biologically either through genetically modification method or Enhanced fertilizer method or by Conventional Selective Breeding method. Biofortification being the most currently globally accepted strategy to combat malnutrition is left or driven by intellectuals and technocrats. Facts and findings are still shared among the same class of people. Even products from findings are moving very slowly to the end or main users. In Nigeria pro-vitamin A cultivar of cassava has been released for over ten years, but till date, more than 50% of rural farmers have not been able to access it.

Iodine is found in soil as iodide, iodate and organic iodine compounds. According to Fuge and Johnson (1986), little iodine is present in the soil solution and most soil iodine is associated with organic matter, clays and oxides of iron and aluminum. The prevalent form of iodine in the soil is iodide (I^-), however, iodate may also be found depending upon the pH and redox conditions because plants take-up iodine in form of iodide anion (Umaly and Poel, 1971; Mackowiak and Grossl, 1999; Zhu *et al* 2003; Blasco *et al* 2008). According to White and Broadley (2001), and Roberts (2006), iodine follows chloride (Cl^-) transport pathway with H^+/anion supporters catalyzing I^- uptake and anion releasing I^- into the xylem.

A chelate is a chemical either organic or inorganic compound composed of a metal ion and a chelating agent. A chelating agent is a substance whose molecules can form several bonds to a single metal ion (Sekhon, 2003). Natural organic chelates are products of microbial activity and degradation of soil organic matter and plant residues. Chelates increase the solubility and supply of micronutrients to plant roots. Chelates complex micronutrients and greatly increase the mass flow and diffusion of micronutrients to the plant root (Havlin *et al*, 2006). Substances released by plant roots are also capable of complexing micronutrients (Havlin *et al*, 2006). Ethylene-diamine-tetra-acetic acid (EDTA) is an inorganic chelate. We also have several commercial organic chelates and unidentified organic chelates naturally lying waste and their potentials untapped.

Periwinkle is seafood common in almost all food markets in Nigeria, especially, South-South and South –Western part of the country (Okpeku *et al*, 2013). Periwinkle effluents are rich in many essential nutrients, including iodine, zinc, magnesium, calcium, potassium and nitrate.

Cassava is a staple food crop in Nigeria. It is the number one food that feed Nigeria (Ikuli and Akonye, 2019) and the seventh that feed the world (Reddy, 2008). It is a food crop that feeds more than 95% of the country's population. It is known as a food security crop (Barrat *et al.*, 2006) because of its ability to trend in many environmentally stressed conditions. Because of its potentials and the population

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percentage of farmers that cultivate it, cassava is excellently fit for biofortification in order to fast track control of the deficient nutrients. There is however, a scarcity of documented information on the performance of natural chelates in the enhancement of iodine biofortification in cassava. The current study has been embarked upon to provide information and performance of natural chelates in the enhancement of iodine biofortification in cassava with a view to produce more nutritious cassava that will contribute to food security.

The objective of the study is to evaluate the ability of different organic chelates to enhance iodine biofortification in cassava applied through foliar.

MATERIAL AND METHODS

The study was carried out at University Park, University of Port Harcourt, Port Harcourt, Rivers state (Lat. 4° 54′ 31″ N and Long.6° 54′ 38″ E). The temperature range from 23°C to 35°C and average annual rainfall of 2293.6mm (www.clima.temps.com.2007), from November 2017 to November, 2018.

Planting Materials and Planting

Cassava cultivars wereobtained from Faculty of Agricultural Teaching and Research Farm, University of Port Harcourt, namely; TME419 and TMS3168/UMUCASS/36 also known as Yellow Root. Cassava stems were cut into a length of 25cm each and was planted 1m by 1m.

Land Preparation and Plot Layout

A total land area of 522m^2 was cleared, ploughed and was partitioned into 30 plots. The plot size was 2m x 5m with ten (10) treatments and three replicates. The distance in-between treatment is 1m and replicates 1.5m apart. The randomized complete block design (RCBD) was used.

Biofortification Material

Hydrochloric acid (HCl), Potassium iodide (KI) source of iodine and Ethylenediaminetetra-acetic acid (EDTA) used as standard chelate were obtained from BENERCO Enterprises Alakahia, Port Harcourt, Rivers State

Bontera; A microbial soil enhancer was sourced from Organico, A division of Amka Products in South Africa used as commercial organic chelate.

Periwinkle extracts were obtained from market women at Omuchiolu Aluu local market. Smoke solution used as local organic chelate was locally prepared from dry wood particles.

Preparation of Fortifying Solution

The Potassium iodide (KI) used as the iodine fertilizer was diluted to 100µg of iodine concentration fortifying solution was prepared in the following steps

The glassware was treated with Hydrochloric acid to remove all traces of contaminants

Potassium iodine (KI) weighing 6.541g was dissolved in 500ml of redistilled water and diluted to 1ml = 10mg of iodine (solution A).

Ten millilitres (10ml) of solution A was diluted in 100ml of redistilled water to get 1ml = 1mg (solution B).

Ten millilitres (10ml) of solution B was diluted in 100ml of redistilled water to give 1ml = 100µg (solution C).

One hundred millilitres (100ml) of solution C was added to 5000ml each of deionized water, 1ml/l Bontera, 1ml/L EDTA, Periwinkle effluents and Smoke solution.

Biofortification Application

The prepared biofortifying solutions were applied through foliar application. The use of knap snack sprayer was employed for this purpose. The application was done at the early tuberization and bulking stage of the cassava plant development (3 months after planting), repeated after 3 weeks and repeated at the later bulking stage (7 months after planting).

Iodine Analysis

The leaves, stem and Root (flesh and peel) from each replicate were collected and analysed for bioavailable iodine content and other nutrients in the root.

The iodine content was determined using leuco crystal violet method from APHA (2012) and Association of Official Analytical Chemists (AOAC,) official methods #920.202; (JAOAC, 1973; 1974).

SAS Software (2012) One-way ANOVA and multiple comparism using LSD were used for the statistical analysis.

RESULTS

Iodine Content

The iodine concentration in the cassava plant ranged from 350µg/g (TME419 root peel; Control) to 23440µg/g(YELLOW ROOT stem; SS + KI). In the edible root flesh, the bioavailable iodine concentration ranged from 790µg/g (YELLOW ROOT; PE + KI) to 7710µg/g (TME419; PE + KI) as presented in table 1.

Mobility and Distribution of Iodine in Cassava Plant

Out of the total iodine absorbed by TME419 control, 31.19% was allocated to leaf, 56.17% to stem, 8.47% to the flesh and 4.17 to the peel. In YELLOW ROOT control, 33.48% of the total iodine in the plant was deposited in the leaf, 60.28% in stem, 3.28% in the edible flesh and 2.96% in the peel. For TME419 biofortified with potassium iodine, 32.55% was partitioned to the leaf, 58.56% to the stem, 7.90% to the flesh and 0.99% to the peel. While for YELLOW ROOT biofortified with KI, 30% was found in the leaf, 54.52% in the stem, 13.43% in the edible flesh and 1.76% in the peel. In TME419 Bontera 26.71% was partitioned to leaf, 48.10% to stem, 21.02% and 4.16% to the peel. In YELLOW ROOT Bontera, 31.54% of the total iodine in the plant was partitioned to leaf, 56.76% to stem, 10.27% in the edible flesh and 1.43% in the peel. For TME419 treated with Bontera plus potassium iodine, 30.59% of iodine was partitioned to the leaf, 55.10% to the stem, 9.29% to the flesh and 5.02% to the peel. While for YELLOW ROOT treated with Bontera plus KI, 31.09% of iodine in the plant was found in the leaf, 55.93% in the stem, 9.57% in the edible flesh and 3.41% in the peel. For TME419 treated with EDTA plus potassium iodide, 32.63% of the total bioavailable iodine in the plant was partitioned to the leaf, 58.79% to the stem, 7.18% to the flesh and 1.40% to the peel. While for YELLOW ROOT treated with EDTA + KI, 33.72% was found in the leaf,

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60.70% in the stem, and 4.04% in the edible flesh and1.54 % in the peel.

Table 1: IODINE (µg/g) DISTRIBUTION IN CASSAVA PLANT

Treatment	TME 419 cultivar				Yellow Root (YR) cultivar			
	Leaf	Stem	Root flesh	Root peel	Leaf	Stem	Root flesh	Root peel
CONTROL	7480	13470	2030	970	10310	18560	1010	910
KI	11460	20620	2780	**350**	6700	12060	2970	390
CHELATES								
EDTA	10100	18180	3900	430	5880	10590	3060	800
BT	5070	9130	3990	790	8810	15860	2870	400
PE	7930	14270	2500	820	11170	20110	1750	760
SS	4280	7700	1950	390	7880	14190	1940	360
CHELATES + IODINE SOURCE								
BT +KI	6090	10970	1850	1000	7210	12970	2220	790
EDTA + KI	8410	15150	1850	360	11170	20110	1340	510
PE + KI	620	11180	**7710**	2130	9120	16420	**790**	3060
SS + KI	8320	14980	2590	820	13020	**23440**	2130	760

NB:*EDTA(Ethylene diamine tetra acetic acid); BT (Bontera); PE (Periwinkle Extract); SS (Smoke Solution)*

For TME419 treated with periwinkle extracts, 31.07% of the total bioavailable iodine was partitioned to the leaf, 55.92% to the stem, 9.80% to the flesh and 3.21% to the peel. While for YELLOW ROOT biofortified with periwinkle extract 33.06% was found in the leaf, 59.51% in the stem, 5.18% in the edible flesh and 2.25% in the peel. For TME419 biofortified with Periwinkle effluents plus potassium iodine, 22.78% was partitioned to the leaf, 41.07% to the stem, 28.32% to the flesh and 7.83% to the peel. While for YELLOW ROOT biofortified with Periwinkle effluents + KI, 31.03% of the total bioavailable iodine was found in the leaf, 55.87% in the stem, 2.69% in the edible flesh and 10.41% in the peel. For TME419 treated with Smoke solution, 29.89% was partitioned to the leaf, 53.77% to the stem, 13.62% to the edible flesh and 2.72% to the peel. While for YELLOW ROOT biofortified with Smoke solution, 32.33% was found in the leaf, 58.23% in the stem, 7.96% in the edible flesh and 1.48% in the peel For TME419 biofortified with Smoke solution plus potassium iodine, 21.15% was partitioned to the leaf, 56.08% to the stem, 9.70% to the edible flesh and 3.07% to the peel. While for YELLOW ROOT biofortified with Smoke solution plus KI, 33.09% was found in the leaf, 59.57% in the stem, 5.41% in the edible flesh and 1.93% in the peel.

In the control, TME419 had a higher accumulation efficiency which made it to have higher quantity of iodine stored in the edible part, and in the biofortified; TME419 still had higher accumulation efficiency potential in periwinkle effluents biofortified. In EDTA biofortified, TME419 had better accumulation efficiency than YELLOW ROOT. For those biofortified with Bontera, TME419 had better accumulation efficiency. For those biofortified with Smoke solution and Smoke + KI, TME419 had higher accumulation efficiency. However, there was no significant difference. Those biofortified with Bontera + KI, YELLOW ROOT was better in their accumulation abilities. For treatment biofortified with EDTA +KI, TME419 had better accumulation efficiency. YELLOW ROOT had better accumulation efficiency in only treatment biofortified with only potassium iodide (KI) and the one biofortified with Bontera +KI. This showed that natural chelates like periwinkle effluents and Bontera enhance iodine mobility and that mobility is easier through the phloem and xylem of TME419 than the phloem and xylem of YELLOW ROOT when using natural chelating agents. TME419 had porous or weak tissues that allow free mobility of substances and as such movement of nutrients applied through the leaves moved without restriction and accumulates in the root. And because of the weak or lack of chelating molecules in its tissues, it lacked the ability to withhold the deposited nutrients in its tissues for a long period of time after being processed to garri. Movement in yellow root was slower because of its chelating potentials that restricted movement of the applied nutrients through its xylem and phloem. This gives yellow root the ability to withhold the nutrients that accumulated in its tissues for a longer period of time after processing to garri.

Table 2: IODINE (µg) CONTENT IN PROCESSED CASSAVA

	TME 419 cultivar						Yellow Root (YR) cultivar					
	Fufu	% US FDA DV 150µg	%WHO 1100µg	Garri	% US FDA DV 150µg	%WHO 1100µg	*Fufu*	% US FDA DV 150µg	% WHO 1100µg	Garri	% US FDA DV 150µg	%WHO 1100µg
CONTROL	320	213	29.09	990	660	60	100	66.67	9.09	495	330	45
KI	*350*	233.33	31.82	1360	906.67	123.64	380	253.33	34.55	1400	933.33	127.27
CHELATES												
EDTA	490	326.67	44.55	1910	1273.33	173.64	390	260	35.45	1500	1000	136.36
BT	500	333.33	45.45	1920	1280	174.55	360	240	32.73	1410	940	128.18
PE	340	226.67	30.91	1300	866.67	118.18	230	153.33	20.91	910	606.67	82.73
SS	**140**	93.33	12.73	570	380	51.82	210	140	19.09	820	546.67	74.55
CHELATES + IODINE SOURCE												
BT +KI	230	153.33	20.91	910	606.67	82.73	300	200	27.27	1090	726.67	99.09
EDTA + KI	320	213	29.09	990	660	90	230	153.33	20.91	910	606.67	82.73
PE + KI	1030	686.67	93.64	**4010**	2673.33	364.55	100	66.67	9.09	410	273.33	37.27
SS + KI	330	220	30	1220	813.33	110.91	270	180	24.55	1040	693.33	94.55

NB:*EDTA(Ethylene diamine tetra acetic acid); BT (Bontera); PE (Periwinkle Extract); SS (Smoke Solution); KI (Potassium iodide);USFDA DV (United State Food and Drug Administration established Daily value); WHO (World Health Organisation)*

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Table 3: IODINE (μg/g) CONTENT IN PROCESSED CASSAVA AFTER 7 MONTHS STORAGE

	TME 419 cultivar	Yellow Root (YR) cultivar
	Garri	Garri
CONTROL	0.00	0.24
KI	0.00	0.10
	CHELATES	
EDTA	0.00	0.11
BT	0.00	0.05
PE	0.00	0.03
SS	0.00	0.10
	CHELATES + IODINE SOURCE	
BT +KI	0.00	0.01
EDTA + KI	0.00	0.00
PE + KI	0.00	0.03
SS + KI	0.00	0.04

NB:*EDTA(Ethylene diamine tetra acetic acid); BT (Bontera); PE (Periwinkle Extract); SS (Smoke Solution);KI(Potassium iodide)*

DISCUSSION

Potentials of Chelates in making nutrient available for Plant uptake

Among the chelating agents, when used individually, Bontera had the highest ability to enhance iodine deposition in the edible flesh in TME419 followed by EDTA and YELLOW ROOT; EDTA had higher value than Bontera, although in both cultivars, there was no significant difference between Bontera and EDTA. They were followed by periwinkle effluents in TME419 and Smoke solution in YELLOW ROOT. There was significant difference at 0.5 level of probability between periwinkle effluents and smoke solution in TME419, but there was no significant difference between Smoke solution and Periwinkle effluents in YELLOW ROOT. When used in combination of iodine source (KI); periwinkle effluents had the highest ability to chelate iodine to the edible portion of the root. There was significant difference at 0.5 level of probability between periwinkle effluents and all other treatments in TME419. However, in YELLOW ROOT; Periwinkle effluents had the least value. Smoke solution followed periwinkle effluents when combined with KI, while Bontera in combination with KI had the highest capability followed by EDTA + KI and Smoke solution.

Bioavailability Issue on Cassava Tuber

The study showed that only about approximately 48- 52% of iodine in cassava root is retained after processing the root to garri and 7.17 – 15.76% in *fufu* as indicated in table 2. This indicates the proneness of iodine to leaching. Sufficient amount of iodine is lost during processing of cassava root to *fufu*, since the process involves soaking the cassava root for days (3-5 days) in water. This revealed that cassava when processed as garri retains more iodine than when processed as *fufu*. In other words, the preparation process of the food determines the iodine bioavailability level. However, in both methods of preparation, the bioavailable iodine in almost all the treatments except *fufu* in Smoke solution was higher than the United State Food and Drug Administration (FDA) established daily value of iodine (150µgd¯¹) as indicated in table 2. In *fufu*, the bioavailable content ranged from 66.67 % (YELLOW ROOT; Control and PE +KI) to 686.67% (TME419; PE +KI) of the United State FDA daily value. In garri it ranged from 273.33% (YELLOW ROOT; PE +KI) to 2673.33% (TME419; PE +KI) of the United State FDA daily value. The study also showed that YELLOW ROOT has the ability to retain iodine for a longer period in storage after processing to garri up to 7 months with appreciable quantity. TME419 retained higher amount of iodine after processing, but lost all at long storage period (7 months) as indicated in table 3. Emilie *et al* (2014) reported that only 37% seaweed iodine is absorbed by the human body. Although, there is no known percentage of iodine that the body absorb or utilizes after consumption of iodized tuber crop or iodine biofortified tuber crops, and this is very difficult because, at often times different constituents of a particular meal also have little iodine. To know the actual percentage of iodine the human body makes use of from a particular quantity, a target group or an individual need to be fed with a known iodine level from a sole source biofortified tuber crop food and the amount that will be wasted through faece and urine is measured. This is because more than 90% dietary iodine is excreted through the urine (Patrick, 2008). Since the human body does not synthesis iodine, whatever is obtained from these wastes can be calculated against the total quantity consumed.

Iodine Biofortified food and Human Health

Human body needs thyroid hormones for proper bone and brain development during pregnancy and infancy (US Department of needs Health and Human Services; National Institutes of Health, 2016). Iodine biofortified cassava will help, prevent or control to an extent women among over190 million Nigerians at reproductive age from causing permanent harm to their fetus during pregnancy that can result to mental retardation, stunted growth and delay in sexual development in babies after birth (USDHHS; NIH, 2016). The Federal government's Dietary Guidelines for Americans (2016) recommends that people should get most of the nutrients needed in their bodies through food. Eating these iodine biofortified cassava products conforms to this recommendation. Eating these products will relief the consumers from developing thyroid cancer, since iodine deficient people have the higher risk of developing thyroid cancer when exposed to radioactive iodine especially children. Iodine biofortified Cassava in Nigeria will also control fibrocystic breast disease (NIH, 2016). Iodine biofortified cassava in Nigeria will eradicate or reduce to a greater level all iodine deficiency induced diseases since cassava is a major food crop that is eaten by over 90% of the populace. Iodine biofortified cassava will to a great extent reduce stillbirth. According to Organic Facts (2017) and U.S. National Institutes of Health (NIH); Office of Dietary Supplements, stillbirths or neurocognitive issues like cretinism can be prevented in

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pregnant women with adequate iodine availability; hence biofortified cassava will help to manage pregnancy. The Federal government of Nigeria with support from International donors spends huge amount of money yearly to control and eradicate polio diseases. Biofortifying cassava which is the number one staple food crop in Nigeria with micronutrients like iodine will help reduce cost of polio management. Consumption of iodine biofortified cassava can reverse dysplasia (Eskin *et al*, 1995; Stoddard *et al*, 2008; Venturi *et al*, 2001). Consumption of these iodine biofortified cassava products can cure and prevent goiter and gastric cancer (Josefsson and Ekblad, 2009; Abnet *et al*, 2006; Behrouzian and Aghdami, 2004). Biofortifying cassava with iodine will help to reduce mental retardation (WHO, 2007; MCNeil, 2006; Felig and Frohman, 2001) in countries like Nigeria, since Cassava is the number one food that feed the country. Human becomes prone to infection and disease attack when there is excess toxicity in the body system, iodine biofortified cassava can help to clean up harmful chemicals like fluoride, lead, mercury and living toxins in the body (Organic Facts, 2017). Cassava contains good amount of carbohydrate, which could lead to diabetes if micronutrients that will convert the sugar to energy is insufficient. Since cassava is a staple which many families especially resource- poor families eat almost on daily basis, iodine biofortified cassava can free them from many health problems that would have resulted from eating a mono- diet.

Iodine Biofortification and the Society

Hardship, poverty, stress and sadness are catalyst of mental retardation and frustration. And these are the common features in our society today. If one is deficient of iodine, encounter with little of these will result to health disaster. Consuming iodine biofortified food on daily basis will help to boost the immune of the individual to resist some of these problems from the society. Iodine biofortified cassava will help to eradicate or reduce to a minimal level all health issues related to iodine deficiency. This is because, cassava as a security food crop outside its ability to survive or adapts to harsh environmental conditions, in Nigeria it is the only food crop that every individual (poor or rich) can afford or have access to at all times.

CONCLUSION

To eliminate the dilemma of iodine deficiency in the world, iodine biofortification should be encouraged. When the chelates were used individually, bontera (commercial organic chelate) did better than EDTA which was used as universal standard chelate for evaluation and comparism, although, there was no significant difference. When used in combination with potassium iodide, periwinkle effluents did better than all and there was significant difference in TME419, but it was the least in YELLOW ROOT. It was followed by Bontera in YELLOW ROOT. Among the chelates used, Bontera was the best because; it was stable when used on both cultivars. In general, all the organic chelates used have good potentials in making micronutrients available for food crop. Among the cultivars used, YELLOW ROOT has the best ability to retain micronutrients after being processed to either *fufu* or garri. The use of organic chelates should also be encouraged by enlightening the farmers the potentials of what is lying waste in their domain. And this will facilitate the drive to organic agriculture, production of healthy and nutritious food products for better food security and healthy society.

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