



Assessment of Natural Chelates to Enhance Zinc Biofortification in Cassava (*Manihot esculenta* Crantz)

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ABSTRACT: The study examined the potentials of some organic chelates to enhance zinc 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 zinc obtained from zinc oxide 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 zinc concentration distribution ranged from 13.627µg/g (YR peel; control) to 85.843µg/g (YR edible root; EDTA +ZnO). In the edible root, the bioavailable zinc ranged from 18.494µg/g (YR; Boontera+ ZnO) to 85.843µg/g (YR; EDTA +ZnO). After processing to garri and fufu, the content ranged from 0.2116mg/100g (TME419; control) to 1.1645mg/100g (YR; Smoke solution + ZnO) in fufu and 1.0178mg/100g (YR, Bontera +ZnO) to 4.494mg/100g (TME419; PE +ZnO) in garri. After seven months storage, TME419 retained Zn in 30% of the treatments, while YR retained Zn in 60% of the treatments. The study revealed that organic chelates have good potentials to enhance zinc biofortification. Periwinkle effluents proved to be better because it retains the nutrient for longer period after processing to garri. YELLOW ROOT proved to be better because it can retain zinc for a longer period. The use of organic chelates should be encouraged for micronutrients sufficiency, sustainable agriculture and food security.

DOI: <https://dx.doi.org/10.4314/jasem.v23i8.13>

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Dates: Received: 19 June 2019; Revised: 22 July 2019; Accepted 12 August 2019

Key words: Organic chelates, Zinc, Bioavailability, Cassava

Zinc (Zn) is an essential micronutrient, in that, it is needed in minute amount but its effects on the human body is astronomical. Zinc is among the micronutrients recognized by the World Health Organisation (WHO) as limited (Ortiz-Monasterior *et al.*, 2007). Zinc deficiency influence about 1.9% of the total burden of diseases caused by major health risks worldwide (WHO, 2002; Hotz and Brown, 2004). It has been reported and proven that nutrients obtained from daily food is far more better than the same nutrients obtained from supplements and as such, increasing Zn availability through biofortification in food crops like cassava that is consumed by more than 90% population in Nigeria and over 2 billion people in the world will be a good strategy to combat Zn deficiency among people that consume cassava as a staple food crop. To make this nutrients sufficient for all, the most recommended tool is biofortification (Welch and Graham, 2000; Graham *et al.*, 2001; Bouis *et al.*, 2003; Graham, 2003; Genc *et al.*, 2005; White and Broadley, 2005; White and Broadley, 2009; Ikuli *et al.*, 2017). For effective achievement of biofortification as a strategy, the rural farmers who produce the bulk of the food consumed should be made the drivers of the

strategy, by identifying natural sources of these nutrients many of which are lying waste among the people for easy application and effective utilization of the natural resources endowed to the environment. Cassava is an important component in the diets of over 190 million people in Nigeria today. Greater percent of its cultivation is by resource-poor families in the rural areas. Cassava is a dietary staple in much of the tropical Africa. Cassava is important because it provides about one fifth ($\frac{1}{5}$) of the total calories human need among those that consume it. According to the USDA (2018), every 100g of cassava root contains; 0.34mg (Zn). Cassava with its potential as a food security crop being cultivated and consumed by more than 90% of the Nigerian population and other part of the world, is in better position to be used in Zn biofortification in order to control the daily rising in chronic diseases cases related to Zn deficiency in the world especially Nigeria and Africa.

The objective of this research is to study the response of cassava and potentials of natural chelates to promote Zn availability in edible root via foliar application.

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MATERIALS AND METHODS

The study was carried out at 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 total rainfall range of 2000-3000mm per annum, from November 2017 to November, 2018.

Planting Materials and Planting: Cassava cultivars were obtained 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 522m² 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), Nitric acid, Zinc oxide (ZnO) source of zinc and Ethylene diamine-tetra-acetic acid (EDTA) used as standard chelate were obtained from BENERCO Enterprise Alakahia, Port Harcourt, Rivers State, Nigeria.

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

Periwinkle effluents 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 zinc oxide (ZnO) used as the zinc fertilizer was diluted to 100µg of zinc concentration fortifying solution was prepared in the following steps:

The glassware was treated with HCl to remove all traces of contaminants

Zinc oxide (ZnO) weighing 6.23g was dissolved in 20ml nitric acid, added deionized water to 1000ml level (solution A):

Five millilitres (5ml) of solution A was diluted in 1000ml of deionized water (solution B):

Ten millilitres (10ml) of solution B was diluted in 100ml of deionized 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).

Zinc Analysis: The leaves, stem and Root (flesh and peel) from each treatment were collected and analysed for bioavailable zinc content and other nutrients in the root.

The zinc content was determined using the Atomic Absorption Spectrophotometer (AAS) at a wavelength of 213.86nm.

SAS Software; One-way ANOVA and multiple comparison using LSD was used for the statistical analysis.

RESULTS AND DISCUSSIONS

Zinc content: The total Zinc concentration in the whole cassava plant ranged from 13.627µg/g (YR Root peel; BT + ZnO) to 85.843µg/g (YELLOW ROOT Edible root; EDTA +ZnO). In the edible root, the bioavailable Zn ranged from 18.494µg/g (YELLOW ROOT; BT + ZnO) to 85.843µg/g (YELLOW ROOT; EDTA + ZnO) as presented in table 1.

Zinc Mobility and Distribution in Cassava Plant: In control TME419, 16.42% of the total bioavailable zinc in the plant remained in the leaf, 25.07% in the stem, 33.69% in the edible root and 24.82% in the root peel. While in YELLOW ROOT control, 22.52% was deposited in the leaf, 34.37% in the stem, 24.82% in the edible root and 18.29% in the root peel. For those treated with only zinc oxide (ZnO), in TME419; 17.045% was deposited in the leaf, 26.016% in the stem, 32.783% in the edible root and 24.156% in the root peel. While YELLOW ROOT treated with ZnO retained 10.696% in its leaf, 16.325% in stem, 42.018% in edible root and 30.961 in its root peel. For those treated with the chelates individually; TME419 treated with Bontera, deposited 12.652% in the leaf, 17.410% in stem, 39.173% in the edible root and 28.864% in the root peel. While YELLOW ROOT retained 20.412% in the leaf, 31.165% in the stem, 27.886% in the edible root and 20.547% in the root peel. For those treated with EDTA, TME419

stored 18.37% in its leaf, 28.04% in stem, 30.86% in edible root and 22.73% in its root peel. While YELLOW ROOT stored 14.23% in leaf, 21.72% in stem, 36.88% in edible root and 27.18% in its root peel. In those that were treated with Periwinkle effluents, TME419 retained 25.1% of the total bioavailable zinc in its leaf, 38.31% in stem, 21.07% in edible root and 15.52% in root peel. While in YELLOW ROOT, 18.55% was in the leaf, 28.31% in the stem, 30.60% in the edible root and 22.54% in its root peel. Those treated with Smoke solution, TME419 had 9.91% deposited in its leaf, 15.14% in stem, 43.15% in edible root and 31.80% in its root peel. While YELLOW ROOT deposited 14.76% in its leaf, 22.52% in stem, 36.11% in edible root and 26.61% in root peel. For those that the chelates were applied in combination with zinc oxide; TME419 when treated with Bontera +ZnO had 15.45% in its leaf, 23.57% in the stem, 35.11% in the edible root and 25.87% in its root peel. While YELLOW ROOT had 29.17% in leaf, 44.53% in stem, 15.14% in edible root and 11.16% in root peel. Those treated with EDTA

+ZnO; TME419 had 19.37% in its leaf, 29.56% in stem, 29.40% in edible root and 21.67% in root peel. While YELLOW ROOT had 19.29% in leaf, 29.44% in stem, 29.52% in edible root and 21.75% in the root peel. For those treated with PE +ZnO; TME419 stored 19.22% of the total zinc in its leaf, 29.33% in stem, 29.62% in the edible root and 21.83% in the root peel. While in YELLOW ROOT, 15.45% was deposited in the leaf, 23.59% in the stem, 29.62% in the edible root and 25.86% in the root peel. For those treated with Smoke solution + ZnO, TME419 retained 15.94% in the leaf, 24.33% in the stem, 34.39% in the edible root and 25.34% in the root peel. While YELLOW ROOT stored 14.86% in the leaf, 22.69% in the stem, 35.96% in the edible root and 26.49% in the root peel. In all the treatments, zinc was evenly distributed. This suggests that because of the vital role zinc plays in the metabolic activities in plant, its mobility was not restricted in anyway. And higher percentage in most of the treatments was deposited in the edible root as desired.

Table 1: Zinc ($\mu\text{g/g}$) 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	28.435	43.401	58.329	42.977	39.192	59.819	43.200	31.831
ZnO	36.177	26.525	69.581	51.270	17.378	45.970	68.269	50.304
Chelates								
EDTA	38.373	58.570	64.453	47.492	22.352	34.116	57.941	42.694
BT	19.279	29.422	59.682	43.976	33.488	51.113	45.750	33.710
PE	30.118	45.970	25.276	18.624	42.459	64.805	70.021	51.595
SS	16.259	24.811	70.736	52.121	29.949	45.712	73.285	54.000
Chelates + Iodine Source								
BT + ZnO	29.686	45.310	67.486	49.726	35.638	54.395	18.494	13.627
EDTA + ZnO	43.066	65.733	65.377	48.173	56.076	85.589	85.843	63.253
PE + ZnO	42.882	65.452	66.094	48.701	31.337	47.829	71.175	52.445
SS + ZnO	33.977	51.860	73.293	54.005	27.510	41.989	66.541	49.030

NB: EDTA (Ethylene diamine tetra acetic acid); BT (Bontera); PE (Periwinkle Effluents); SS (Smoke Solution); ZnO (Zinc oxide)

In control TME419 had better accumulation efficiency, the potential to absorb and deposit Zn in the edible root than YELLOW ROOT. TME419 still had higher accumulation efficiency than YELLOW ROOT when they were treated with ZnO. When they were treated with Bontera, TME419 had higher accumulation efficiency. When they were treated with EDTA, TME419 still had better accumulation efficiency but when they were treated with Periwinkle effluents, YELLOW ROOT had higher accumulation efficiency. When they were treated with Smoke solution, YELLOW ROOT had higher accumulation efficiency. When the cultivars were treated with combinations of chelates and source, TME419 had higher accumulation efficiency when treated with; BT +ZnO and SS + ZnO, while YELLOW ROOT had higher accumulation efficiency when treated with EDTA +ZnO and PE + ZnO. This suggests that Zn

mobility is easier through the phloem and xylem of TME419 than passing through the phloem and xylem of YELLOW ROOT. The study also showed that all the chelates facilitated the mobility of Zn except Bontera when used in combination with ZnO. Organic chelates enhance Zn mobility and accumulation in YELLOW ROOT than inorganic chelates. TME419 had flexible 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 retain the deposited nutrients in its tissues for a long period of time after being processed to garri except those chelated with Periwinkle effluents and Bontera as presented in table 3. Movement in yellow root was slower except with aid of organic chelates, because of its chelating potentials, YELLOW ROOT

was able to store Zn that accumulated in its tissues for a longer period of time after processing to garri in those treated with BT, ZnO, PE, EDTA + ZnO, BT + ZnO and even the untreated (control) as indicated in table 3.

Potentials of Chelates in making nutrient available for Plant uptake and storage: Smoke solution had the highest influence in retaining Zn in the edible root in both cultivars when applied as single treatments, and was followed by ZnO in TME419 and Periwinkle effluents in YELLOW ROOT. When used in combination with ZnO; in TME419; SS + ZnO had the highest influence followed by BT + ZnO and the least was EDTA + ZnO. While in YELLOW; EDTA + ZnO had the highest ability to deposit Zn in the edible root followed by PE + ZnO and the least was BT + ZnO. There was significance difference at 5% level of probability. Among the chelates, smoke solution seems to have the best capability to chelate Zn for cassava plant, but lacks the potential to store the Zn in processed cassava (garri) for long period of time like Bontera and Periwinkle effluents.

Bioavailable Issue: Because of the involvement and vitals roles Zn plays in plant metabolic activities in the whole plant growth, development and productivity, the amount of bioavailable Zn often deposited in the edible portion of the plant for human consumption is affected. However, biofortification helped to elevate this value. From the amount of bioavailable Zn after processing to *fufu* and garri, the amount retained in many treatments exceeded the Harvest Plus target for Zn availability. The bioavailable Zn in cassava processed to *fufu* ranged from 0.2116mg/100g (TME419; Control) to 1.164mg/100g (YELLOW ROOT; SS+ ZnO). These represent 2.116% of the average UK guidance reference nutrient intake (RNI) and 2.015% of the average US Recommended Daily Allowance (RDA) to 11.645% of the average UK RNI and 11.090% of the mean US RDA. In garri the bioavailable Zn ranged from 1.0178mg/100g (YELLOW ROOT; BT + ZnO) to 4.4941mg/100g(TME419; PE +ZnO) and these values represent 10.178% of the average UK RNI and 9.693% of the average US RDA to 44.941% of the average UK RNI and 42.801% of the average United State Recommended Daily Allowance. However, comparing these values to the USDA 1 Released April, 2018 which said that every 100g of cassava contains 0.34mg of Zn, the bioavailable Zn in both cultivars processed to garri in all the treatments were higher. When processed to *fufu*, 70% of the whole treatments in TME419 were higher, only 30% (Control, PE and EDTA + ZnO) were lower and 100% treatments in YELLOW ROOT were higher than the USDA, 2018

value released. Fifty (50%) of the treatments in TME419 (ZnO, Smoke solution, Smoke solution +ZnO, Periwinkle extracts + ZnO and EDTA), when processed to garri exceeded the HarvestPlus Zn target for cassava which is 34mg/kg (3.4mg/100g)

Zinc Biofortified Food Crops and Human Health: This study showed that illness and diseases induced by Zn deficiency in country like Nigeria and other countries that consume cassava as a staple can be brought to a halt. Consuming Zn biofortified cassava will help reduce the issue of infertility in both male and female. The male reproductive system need high amount of Zn to increase the testosterone levels and for the male prostate to perform optimally. The female reproductive system requires zinc for the growth of the oocyte (egg) (Poliquin, 2012). Inadequate Zn causes premature egg and ovulation impeded in female and low sperm count in male. Tumour growth infection is on the increase on daily basis; tumour results from inability of damaged prostate cells to accumulate zinc and thus enhance growth of cancer cells. Biofortifying cassava with Zn will eliminate this menace in Nigeria since it is a major consumed staple. Research has proved that many Zn supplements contain cadmium (Cd), and high amount or frequent intake of cadmium (Cd) causes kidney failure. Consuming Zn biofortified cassava frees the populace from the risk of kidney failure resulting from consumption of Zn supplements. Zinc fights cancerous cells (Poliquin, 2012; Nordqvist, 2017); and consuming Zn biofortified cassava will help to reduce cancer disease; a disease that no proper treatment solution has been achieved. Zinc biofortified cassava will eradicate diabetes in the society. Zinc helps insulin to function efficiently in converting blood sugar to energy and other metabolic activities. Zinc help to maintain the health of cardiovascular cells and endothelium for efficient elimination of cholesterol and inflammation, and reduce the risk of heart diseases (Poliquin, 2012; Nordqvist, 2017). Because of the antioxidant property of zinc, consuming Zn biofortied cassava, will help neutralize free radical bonding to what we consume that are inside the body that can cause damage to the body system and also remove heavy metals from the brain to avoid building up in the tissue to cause damage (Poliquin, 2012). Biofortifying cassava with Zn will eradicate stunting in the society. Biofortifying cassava with Zn will produce citizens with strong immune systems, because Zn is essential for growth. This will also help in age related ill health and muscular problems. Zinc is necessary for the synthesis of DNA and is also needed for fast healing of wounds. Biofortifying cassava with Zn in Nigeria will make Zn availability sufficient for greater percentage of the populace.

Table 2: Zinc (mg/100g) Content In Processed Cassava

TME 419 cultivar		Yellow Root (YR) cultivar										
Treatment	Fufu mg/100g	%UK RNI 7-13mg (10mg)	% USRDA 8-13mg (10.5mg)	Garri mg/100g	%UK RNI 7-13mg (10mg)	US RDA 8-13mg (10.5mg)	Fufu mg/100g	%UK RNI 7-13mg (10mg)	US RDA 8-13mg (10.5mg)	Garri mg/100g	%UK RNI 7-13mg (10mg)	US RDA 8-13mg (10.5mg)
Control	0.2116	2.116	2.015	1.7632	17.63	16.792	0.6760	6.76	6.381	2.3912	23.912	22.773
ZnO	0.835	8.35	7.952	4.1749	41.749	39.761	0.8875	8.875	8.452	4.0961	40.961	39.010
Chelates												
EDTA	0.8545	8.545	8.138	3.8677	38.677	36.835	0.7752	7.752	7.383	3.8821	38.821	36.972
BT	0.7759	7.759	7.390	3.2825	32.825	31.262	0.5948	5.948	5.665	2.5163	25.163	23.965
PE	0.3286	3.286	3.130	1.5166	15.16	14.438	0.9103	9.103	8.670	4.2013	42.013	40.012
SS	0.8488	8.488	8.084	4.2442	42.442	40.421	0.8794	8.794	8.375	4.3971	43.971	41.877
Chelates + Iodine Source												
BT + ZnO	0.8107	8.107	7.721	2.8345	28.345	26.995	0.4629	4.629	4.409	1.0178	10.178	9.693
EDTA + ZnO	0.2192	2.192	2.088	2.9426	29.426	28.025	0.6163	6.163	5.870	3.6057	36.057	34.34
PE + ZnO	0.6062	6.062	5.773	4.4941	44.941	42.801	0.5682	5.682	5.411	3.0612	30.612	29.154
SS + ZnO	0.9562	9.562	9.107	3.7830	37.83	36.029	1.1645	11.645	11.090	2.7950	27.950	26.619

NB: EDTA (Ethylene diamine tetra acetic acid); BT (Bontera); PE (Periwinkle Effluents); SS (Smoke Solution); ZnO (Zinc oxide); US RDA (United State Recommended Daily Allowance); UK RNI (United Kingdom, Reference nutrient intake). In YELLOW ROOT, the bioavailable Zn after processed to garri 50% (ZnO, EDTA, PE, EDTA + ZnO and Smoke solution) of all the treatments exceeded the Harvest Plus target for zinc in cassava.

Table 3: Zinc (mg/100g) content in processed cassava after 7 months storage

Treatment	TME 419 cultivar	Yellow Root (YR) cultivar
	Garri mg/100g	Garri mg/100g
CONTROL	0.000	0.283
ZnO	0.000	0.130
CHELATE S		
EDTA	0.000	0.000
BT	0.054	0.005
PE	0.001	0.013
SS	0.000	0.000
CHELATE S + IODINE SOURCE		
BT + ZnO	0.000	0.683
EDTA + ZnO	0.000	0.199
PE + ZnO	0.334	0.000
SS + ZnO	0.000	0.000

NB: EDTA (Ethylene diamine tetra acetic acid); BT (Bontera); PE (Periwinkle Effluents); SS (Smoke Solution); ZnO (Zinc oxide);

Organic Chelate Biofortification and Food Security:
 The use of natural sources of nutrients in biofortification frees the soil and environment from pollutants and contaminants often introduced by inorganic sources of nutrients that are not utilized by the plant. These natural chelates are rich in many essential macro and micro nutrients needed by plant and nutrients that have chelating properties like sodium (Na). For example, every one litre of periwinkle effluents contains; 153.13mg (K), 55.30mg(Ca), 207.50mg(Mg), 9.09mg(P),

193.75mg(Na), 2.18mg(I), 12.80mg(NO₃), 0.61mg(Zn), 0.9mg(Cu), 0.2mg(Mn) and 1.90mg(Fe) with a pH of 6.5 which favours the absorption of all nutrients. In every one litre of Smoke solution you have; 0.63mg (K), 1.43mg(Ca), 0.42mg(Mg), 2.18mg(NO₃), 1.63mg(Na), 14.70mg(P), 1.38mg(Fe), 0.08mg(Cu) and 0.28mg(Zn) or more depending on quantity of dry wood particles used with a pH of 5.36 which is optimal for all nutrients absorption. In every 2ml/l of Bontera (microbial soil enhancer) a commercial organic chelate; 267.25mg(P), 19mg (K),

3.49mg(NO₃) 3mg(Ca), 0.81mg(Mg), 7.51mg(Na), 1.47mg(Fe), 0.4mg(Zn), 0.08mg(Cu) and 1.02mg(I) with pH of 6.71, which is good for many nutrients and optimal for all in high acidic soil. They all have good chelating agents that bond nutrients and make them available for plant uptake and this will increase productivity. When organic chelates and inorganic sources were applied individually, including a universal standard inorganic chelate (EDTA), Smoke solution influenced Zn bioavailability these more than other sources in both cultivars. When used in combination with ZnO, it was still the second to the highest. After processing cassava to *fufu* and garri, local organic chelates still retained the highest amount of Zn (Smoke solution + ZnO in *fufu* and Periwinkle effluents + ZnO in garri). Among the chelates used, Periwinkle effluent was the best because; it helps to store nutrients for longer time and was stable when used on both cultivars in combination with ZnO. In general, all the organic chelates used have good potentials in making micronutrients available for food crop. This has proved the organic chelates to be better, because they are environmental friendly, cost effective and easy application by all farmers. They also enhanced nutrients storage in food for a longer period and renew vigor of planting materials. Among the cultivars used, YELLOW ROOT has the best ability to retain micronutrients after being processed to either *fufu* or garri for a longer period. The method of processing and the end product in which cassava is processed also determine the level of bioavailability of Zn.

Zinc biofortification and Availability of Planting Material in Cassava: Zinc (Zn) is involved in many enzymatic activities. Zinc is vital in the synthesis of tryptophan, a component of some proteins compound needed for the production of growth hormones, particularly auxin like Indole Acetic Acid (IAA) (Havlin *et al*, 2006). In plants Zn is used in chlorophyll formation, carbohydrate and conversion of starch to sugar. It helps plants to build cold/frost resistance immune. Biofortifying cassava with zinc provides sufficient zinc for all these metabolic activities. The ability of Zn to generate auxin prolongs the shelf-life of the cassava planting material. This helps to delay senescence in cassava plant and enhance yield and makes more stems available for reproduction. The vigour of planting materials is renewed yearly after every Zn biofortification exercise and this enhance productivity.

Conclusion: To build a healthy society free of chronic diseases with food security, rural farmers who produce the bulk of food consumed should be the drivers of biofortification to conquer malnutrition. The use of

organic chelates should also be encouraged by proper orientation of natural resources abound in our environment for judicious utilization of the potentials of what is lying waste in the environment. And this will enhance clean and healthy environment, production of healthy, nutritious and longer shelf-life food products for better food security and healthy society.

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