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Field Performance of Xanthosoma sagittifolium L. Schott Minitubers Grown Under the Influence of Poultry Manure and NPK Fertilizers: Changes in Content of Some Secondary Metabolites

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Abstract

The response of white and red cultivar (cv) cocoyam *(Xanthosoma sagittifolium* (L.) Schott) minituber seeds to different rates of poultry manure (PM) and NPK (20:10:10) fertilizer was studied under field conditions in the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (outskirsts of Yaounde) Centre region, Cameroon. Treatment combinations comprising of three rates each of poultry manure (0, 20 and 30t ha^{-1}) and NPK fertilizer (0, 120 and 150 kg ha^{-1}) were factorized and fitted into a randomized complete block design with three replicates. Physico-chemical analysis of all treatment plots revealed clay loam textures and poultry manure treatment plots significantly ($P<0.05$) increased soil pH, organic content, total carbon and cation exchange capacity while NPK treatments significantly ($p<0.05$) increased available phosphorus compared to the control treatments. Poultry manure(30t ha^{-1}) treatments significantly ($P<0.05$) enhanced plant height, leaf number and leaf area in both cultivars after 6 months of growth as compared to all other treatments. Under the same treatments of poultry manure (30t ha^{-1}) the white cultivar plants recorded the most significant mean yield parameters after 9 months of growth. The average tuber number per plant (8), tuber weight (250g), tuber length (14cm) and tuber girth (18.5cm) compared to the red cultivar average yield parameters: tuber number per plant (5.7), tuber weight (124.7g), tuber length (8cm) and tuber girth (14.5cm). Biochemically the application of various treatments of poultry manure and NPK fertilizers significantly ($P<0.05$) influenced the changes observed in the secondary metabolites studied. Two months after planting, NPK (150kg ha^{-1}) treatments showed the most significant phenolic content (1.22±0.13mg eq catechin.g^{-1} FW) and flavonoid content (1.08±0.16mg g^{-1} FW) in white cv cocoyam plants. After 6 months of growth, the poultry manure (30t ha^{-1}) treatments recorded the most significant protein content (5.04±0.38mg eq BSA.g^{-1} FW) and peroxidase activity (4.89±1.36 UE min^{-1}) in white cv cocoyam plants while red cv cocoyam plants had the most significant glucanase activity (9.33±1.17 mg eq glucose g^{-1} FW). NPK (150kg ha^{-1}) treatments in white cv cocoyam plants recorded the most significant polyphenol oxidase activity (4.99±0.10 in D330 nm^{-1} min^{-1} UE^{-1} g^{-1} FW) 6 months after planting.

Keywords: *Xanthosoma sagittifolium* L. Schott; Minitubers; cation exchange capacity; Poultry manure; NPK fertilizers; yield parameters and secondary metabolites.

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1. Introduction

Cocoyam (*Xanthosoma sagittifolium* L. Schott, Araceae) is a herbaceous plant cultivated in tropical and subtropical regions for its edible tubers and leaves. Cocoyam tubers and leaves are essentially rich in sugars, proteins, vitamins and mineral salts (Sefa & Sackey, 2004). It covers the food needs of more than 200 million persons in the tropical and subtropical regions and more than 400 million persons worldwide (Onokpise *et al.,* 1999). It occupies the sixth position worldwide with an annual production of 0.45 million metric tonnes (FAO stat, 2006) and the second in Cameroon after cassava (*Mannihot esculenta*) in terms of tuber production. The National Programme on Roots and Tubers (NPRT) was launched in Cameroon in 2005. Annual production of cocoyam has since increased from 1,240,037 tons in 2005 to 2,000,000 tons (NPRT stat, 2013) with projections of 3000,000 tons by 2020.

In Cameroon, the unavailability of healthy seeds to farmers has affected the National production rate. The infestation of seeds is due to several viral, fungal and bacterial diseases (FAO stat, 2008;Chen & Adams, 2001;Perneel *et al.,* 2006).Otherwise, the lack of cocoyam seeds can be justified by the fact that cocoyam usually

propagates vegetatively from tuber fragments, this increases the dissemination of many pathogens which cause rot disease in cocoyam such as *Pythium myriotylum* (Perneel *et al.,* 2006;Boudjeko *et al.,* 2005) ,*Fusarium oxysporum* and *Fusarium solani* (Ubalua *et al.,* 2008;Anele & Nwawuisi, 2008) and Dasheen mosaic virus that is found in the leaves, corm and cormels (Chen & Adams, 2001). To overcome such problems, efforts have been made to improve the application of tissue culture technology to cocoyam production through *in vitro* regeneration of cocoyam plants using biotechnology (Omokolo *et al.,* 2003).Nowadays the production of cocoyam minitubers can be considered as a revolution like the production of potato minitubers. Cocoyam minitubers could be used as an alternative for basic seeds produced through plantlets obtained *in vitro* (Djeuani *et al.,* 2014).

Inspite of the important role played by cocoyam in feeding, another major factor which accounts for low production is the increasing decline in soil fertility levels and lack of soil management practices for continuous cocoyam cultivation(Agbede *et al.,* 2014). Low activity clays characterize tropical soils and the magnitude of nutrient depletion especially in Africa's agricultural production systems is enormous(Stoorvogel, 1990). This depletion of soil fertility is widely recognized as the major cause of low food crop production in sub-Saharan Africa (Sanchez & Jama, 2000). External fertilising agents to agricultural production systems include mineral fertilisers such as urea, NPK, ammonium nitrate, sulfates, and phosphates; organic fertilisers such as animal manures, composts, and biosolids. The use of mineral fertilizers in sub-Saharan Africa is limited by the lack of purchasing power and scarcity of the product in the smallholder sectors while their continuous use can also lead to a decline in soil organic manure (SOM) by enhancing its decomposition (Giller *et al.,* 2009), making SOM a critical nutrient source. The use of organic and mineral fertilizers are the two major and common ways in which soils are managed since the extinction of shifting cultivation as well as reduction in bush fallow periods (Makinde *et al.,* 2011). The impact of increased use of mineral fertilizers on crops has been high but the resulting soil physical degradation, increased soil acidity and soil nutrient imbalance, resulting in reduced crop yield (Ojeniyi, 2000;Mbah & Mbagwu, 2006) escalating cost and unavailability of mineral fertilizers (Surge *et al.,* 2011) have drawn the attention of researchers back to the use of organic manures. Organic manures contain humic substances which play a vital role in soil fertility and plant nutrition. Plants grown on soils which contain adequate humin, humic adds (HAs), and fulvic adds (FAs) are less subject to stress, are healthier, produce higher yields; and the nutritional quality of harvested foods and feeds are superior. These organic manures like poultry manure are cheaper, readily available and affordable for soil fertility management and improvement in crop yield. The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids(Baneerjee & Mondal, 2012). Different manures (fish, pigeon and cow) and synthetic fertilizers (nitrogen) have been shown to influence some biochemical activities like phenolic constituents of plants (Tugba *et al.,* 2014).The quantity of phenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices postharvest storage and processing (Chandrasekara & Kumar, 2016). Cocoyam responds very well to input of fertilizer whether organic or inorganic as reported by several workers (Hota *et al.,* 2014;Ogbonna & Nwaeze, 2012;Ojeniyi *et al.,* 2013). It has a high requirement for potassium like all other tuber crops (Obigbesan, 1980). In tuber crops, potassium plays a vital role in the movement of sugars produced in the leaf by photosynthesis to the tubers where the sugars are converted to starch (Abd El Latif *et al.,* 2011) Potatoes require high amounts of potassium (K) and nitrogen (N) fertilizers for optimum growth, production and tuber quality (Al- Moshileh *et al.,* 2005).

Against the above background, this study was carried out to: (1) Determine the seed potential of the white and red cultivar cocoyam (*Xanthosoma sagittifolium* L. Schott**)** minitubers under different fertilizer treatments (Poultry manure and NPK 20:10:10) (2) Evaluate the effects of these fertilizer treatments on the changes in the content of some secondary metabolites in the leaves of white and red cocoyam (*Xanthosoma sagittifolium***)** cultivars during growth.

2. Materials and methods

2.1. Site location

A field trial was conducted to study the performance of Xanthosoma sagittifolium minituber seeds as influenced by poultry manure and NPK fertilizer during the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (Outskirsts of Yaoundé) Centre region, Cameroon, located at latitude 3°49′52.54″N and longitude 11°27′15.79″E and 714 m above sea level. The area is characterized as a humid rainforest zone and the soil is clay loam. The total annual rainfalls for 2017 was 1902.8 mm while the total rainfalls during the period of experimentation (April to December) for 2017 was 1775.6 mm.

2.2. Materials

The planting material consisted of white and red cultivars of X. sagittifolium minituber seeds of mean weight 38g produced from acclimatised vitroplants under the shed in the plant physiology Laboratory of the Higher

Teachers Training college (HTTC), University of Yaoundé I. Yaoundé, Cameroon (Djeuani et al., 2014, 2017). The Poultry manure was obtained from Henri et Freres Poultry farm Yaoundé, Cameroon while the NPK fertilizer (20:10:10) was obtained from the fertilizer unit of the Centre Regional Delegation of Agriculture and Rural Development, Yaoundé, Cameroon.

2.3. Experimental design, treatments, soil and morphological analysis during growth and yield of cocoyam plants

The experiment was a 4×2 factorial arrangement in a randomized complete block design and replicated three times. The site was ploughed, ridged and marked out into two main blocks, one for each cultivar. Each of these main blocks was further subdivided into 3 sub blocks which represent the three replicates. Each sub block was divided into five experimental plots, thus a total of fifteen plots were used for cultivar. Each gross plot measured 4m×3m (12 m^2) with a net plot of 2m×2m. Soil samples were collected with soil auger at a depth of 0 to 20 cm from different locations of the site and bulked into composite sample. The composite soil sample was air dried, passed through 2 mm sieve, and then analyzed for its physicochemical properties before planting and after harvest (Table1). Particle size distribution was performed to determine the soil textural class using hydrometer method as described by Gee & Bauder, 1986. Soil pH was determined in distilled water and potassium chloride solution using pH meter (Mclean, 1982). Soil nitrogen was determined by Macro-Kjeldahl digestion method (Bremner, 1982). Exchangeable Ca and Mg were obtained by the complexometric titration method of Chapman, 1982, and exchangeable Na and K were determined by flame photometer. Cation Exchange Capacity (CEC) was determined by modified ammonium acetate method of Chapman, 1982 while available P determined by Bray II method (Bray, 1945). The treatments comprised three rates each of application of Poultry Manure (0, 20, and 30 t ha-1) and NPK fertilizer (0, 120, and 150 kg ha-1). A total of fifteen treatment combinations and three replications were used. The Poultry manure was incorporated into the soils on the experimental plots in a single application based on the treatment combinations, at 2 weeks before planting to ease decomposition, while the NPK fertilizer was applied to the cocoyam stands according to treatment allocation at 4 weeks after planting (WAP) using the ring placement method. Each minituber was planted per hole at a depth of 15 cm and at a spacing of 0.5 m x 1.0m resulting to about twenty-five plants per plot and a total of 375 plants per cultivar. All plots were kept weed free by manual weeding. Five cocoyam plants were randomly selected from each of the net plots, tagged and then used for the determination of average plant height (cm), average number of leaves, and average leaf area at 1, 2, 3,4,5,6,7, and 8 months after planting (MAP). Yield parameters like length (cm) and girth (cm) of tubers, number of tubers per plant, and tuber weight (g) were assessed after 9 months at physiological maturity. The leaf area was determined using the formula of (Biradar, 1982) as: Leaf Area of Cocoyam = 0.917 (LW). Where L and W are length and width of the cocoyam leaf.

2.4. Biochemical analysis

Biochemical analyses consisted of extraction and assay of total soluble proteins, peroxidases, β-1,3 glucanases, Polyphenoloxidases, Total Phenol content and flavonoid content in the leaves each month for 6 months after planting.

2.4.1 Protein Content

Proteins were extracted according to the modified method of Pirovani, 2008. 1g of leaves were ground in chilled mortar with 5 mL of Tris- Maleate buffer (10mM pH 7.2). The crude homogenate was centrifuged for 25 min at 10000 g and 4°C after incubation. The supernatant was removed and used as crude extract for protein and enzymes assays. Proteins were quantified according to method described by Bradford, 1976. 10 µl of extract were added to 490 µl of distilled water and 500 µl of Bradford reagent. The mixture was incubated at 25°C in darkness for 15 min and OD of protein determined at 595nm. The protein content was expressed in mg-equivalent of BSA per Fresh Weight (Bradford, 1976).

2.4.2 Peroxidase Assay

Peroxidase assay in protein extract was done according the modified method of Baaziz et al., 1994. The reaction medium contained 925 µl of Tris- Maleate buffer (10mM pH 7.2, containing 1g gaïacol), 25 µl of protein extract and 50 µl of H_2O_2 (10%). The mixture was incubated for 3min at 25°C. Peroxidase activity was determined following the formation of tetragaïacol at 470nm. Peroxidase activity was expressed in enzyme units per Fresh Weight (FW).

2.4.3. β-1,3 Glucanase Assay

The activity of β-1,3-glucanases was evaluated in the protein extract according to the modified method described by Leelasuphakul, 2006. The reaction mixture contained 90 µl of sodium acetate buffer (0.1M, pH 4 containing 25mg/L of Laminarin), 10 µl of protein extract incubated for 10 min at 40°C. 200 µl of 2M HCl is used to stop the reaction. The OD was read at 540nm. β-1,3-glucanase activity was expressed in µmole of glucose released/min/g of Fresh Weight.

2.4.4. Polyphenol oxidase Assay

Polyphenol oxidase assay was determined in the protein extract according to the method of Vankammenn & Broumer, 1964. The reaction mixture contained 500 µl of phosphate buffer (0.66M, pH 7), 150 µl of catechine and 35 µl of protein extract incubated at 25°C for 30 s. The change in absorbance was read after 5min at 330nm. Polyphenol oxidase activity was expressed in D_{330nm} /min/UE/g of Fresh Weight (FW)

2.4.5. Total Phenol Content

Phenolic compounds were extracted according to the modified method of Boudjeko et al., 2007. 1 g of leaves of cocoyam was ground in chilled mortars with 5 ml of 80% (v/v) methanol at 4°C. After incubation, tubes were centrifuged thrice at 7000 g for 30 min, supernatants were collected each time. Mixture of the three supernatants constituted the crude extract. Total phenols were quantified using the method described by Marigo, 1973. 10 µl of alcoholic extract were added to 500 µl of distilled water, Folin-Ciocalteu reagent (75 µl) and 500 µl of sodium carbonate (20%). The mixture was incubated at 40°C for 20 min and the blue color was determined at 760 nm. The content of soluble phenolic was expressed in mg-equivalent of gallic acid per Fresh Weight (FW).

2.4.6. Flavonoid Content

Flavonoid content was determined according to the modified method of Kramling, 1969.The reaction medium contains 400 µl of phenol extract, 200µl of HCl (50%), 200 µl of Formaldehyde (8mg/L) incubated at 4°C for 15min. The mixture was centrifuged at 3000 g for 5min and the supernatant collected. The supernatant was then used to assay non flavonoid compounds according to the method described by Marigo, 1973. Flavonoid content was then determined using the following formula:

$$T_{flavonoids} = T_{total\ phenol} - T_{non\ flavonoids}$$

3. Results

3.1. Soil analyses before planting and after harvest

Physical analysis of the sand, clay and silt content of the soil on the different treatment plots before planting and after harvest on our experimental farm illustrated a clay loam textural class. (Table 1). Chemically, before planting the soil was slightly acidic (pH 5.6) and both poultry manure treatments (20t.ha^{-1} and 30t ha^{-1}) after harvest significantly reduced soil acidity (pH 6.6 and pH 6.9) (Table 2). Poultry manure treatments (20t ha^{-1} and 30t ha^{-1}) significantly increased organic and carbon content and also recorded the highest cation exchange capacity values as compared to the other treatments. (Table 2).

3.2. Evaluation of the growth parameters of X. sagittifolium plants under different treatments

All growth parameters (plant height, number of leaves plant^{-1} and leaf area) analysed were generally more significant in the white cultivar cocoyam (white cv) plants than in the red cultivar cocoyam (red cv) plants for all treatments. The poultry manure treatments (30t ha^{-1}) showed the most significant increase in all growth parameters analysed from two months after planting to six months after planting among all treatments meanwhile within the same growth period the control treatments (0t of Poultry manure ha^{-1} and 0kg of NPK ha^{-1}) recorded the least increase in average plant height and leaf area for both white and red cultivars of the cocoyam plants. At six months after planting white cv cocoyam plants treated poultry manure (30t ha^{-1}) showed the most significant growth in terms of average height (86cm) (Table 3, Fig 1.) followed by an average height of 77cm for cv red cocoyam plants treated with poultry manure(30tons/ha). (Table 4, Fig 1.). The cv red cocoyam plants showed no significant difference in the average number of leaves for all the different treatments throughout the growth period (Table 4.) meanwhile a significant average number of leaves was recorded (6) after six months of growth for white cv cocoyam plants treated with poultry manure (30t ha^{-1}) (Table 3). White cv plants treated with poultry manure (30t ha^{-1}) after six months of growth recorded the most significant average leaf area (0.88m^2) (Table 3) while red cv cocoyam plants treated with poultry manure (30t.ha^{-1}) recorded an average leaf area of 0.069m^2 after six months of growth. (Table 4)

3.3. Evaluation of yield parameters of cocoyam under different treatments

After a period of 9 months the yield parameters were assessed. The four yield parameters (tuber number plant^{-1}, tuber weight, tuber length and tuber girth) analysed after harvest were generally greater in white cv cocoyam plants than in red cv cocoyam plants for all treatments. Poultry manure treatments (30t ha^{-1}) showed the most significant yield parameters while the control treatments (0tons of Poultry manure ha^{-1} and 0kg of NPK ha^{-1}) had the least yield parameters for both cultivars. White cv plants treated with poultry manure (30t ha^{-1}) recorded an average tuber number per plant of 8, an average tuber weight of 250g, an average tuber length of 14cm, and an average girth of 18.5cm (Table 5 and Fig 2) meanwhile red cv plants treated with poultry manure (30t ha^{-1}) recorded 5.7 as average tuber number per plant, 124.7g as average tuber weight, 8cm as average tuber length and 14.5cm as average tuber girth.(Table 6 and Fig 2).

3.4. Evaluation of some biochemical parameters

After 6 months of growth, the treatment poultry manure (30 t.ha^{-1}) recorded the most significant protein content (5.04±0.38mg eq BSA g^{-1} FW) for white cv cocoyam plants followed by the same treatments for red cv (4.11±0.52mg eq BSA g^{-1} FW). The Control White cv plants (0 tons of Poultry manure ha^{-1} and 0 kg of NPK ha^{-1}) had the least protein content (2.56±0.29 mg eq BSA g^{-1} FW) while there was no significant difference for the Control and NPK (150kg ha^{-1}) treatments in red cv plants after 6 months of growth. (Fig.3A).

Peroxidase activity increased in the three treatments (Control, Poultry manure 30t ha^{-1} and NPK 150kg ha^{-1}) for white cv plants from 2 months after planting to six months after planting with Poultry manure 30t ha^{-1} recording the most significant value of 4.89±1.36 UE min^{-1} at 6 months. The Control, and NPK 150kg ha^{-1} treatments for red cv plants recorded an increase in peroxidise activity between 2 and 4 months after planting, followed by a significant decrease at 6 months (Fig.3B)

The most significant value of glucanase activity was observed in red cv cocoyam plants treated with Poultry manure (30t ha^{-1}), 9.33±1.17 mg eq glucose g^{-1} FW meanwhile white cv cocoyam plants treated with Poultry manure (30t ha^{-1}), recorded 7.36±0.28mg eq glucose g^{-1} FW after six months of planting. The least value for glucanase activity was seen in red cv plants treated with NPK (150kg ha^{-1}), 1.34±.24 mg eq glucose g^{-1} FW after 4 months of planting (Fig.4A).

After 6 months of growth, polyphenol oxidase activity was most significant in Cv white cocoyam plants treated with NPK (150kg ha^{-1}), 4.99±0.10 in D330 nm^{-1} min^{-1}UE^{-1}g^{-1} FW while the red cv Control, Poultry manure (30t ha^{-1}) and NPK (150kg ha^{-1}) treatments showed no significant difference in polyphenol oxidase Red cv cocoyam plants treated with Poultry manure (30t ha^{-1}) recorded a polyphenol oxidase activity of 4.39±0.33 in D330 nm^{-1} min^{-1} UE^{-1} g^{-1} FW after 4 months of planting (Fig.4B).

NPK (150kg ha^{-1}) treatments recorded the most significant values in phenol contents in both cultivars, white cv cocoyam plants had 1.22±0.13 mg eq catechin g^{-1} FW and red cv cocoyam plants had 1.12±0.11mg eq catechin g^{-1} FW after 2 months of planting. The white cv cocoyam plants showed no significant difference in phenolic contents in the Control, Poultry manure (30t ha^{-1}) and NPK (150kg ha^{-1}) treatments after 4 months of planting (Fig.5A).

Flavonoid content was greatest at 2 months after planting in red cv cocoyam plants treated with NPK (150kg ha^{-1}), 1.08±0.16mg g^{-1} FW while white cv cocoyam plants treated with Poultry manure (30t ha^{-1}) also recorded a significant flavonoid content value of 0.96±0.03mg g^{-1} FW after 6 months of growth. The least flavonoid content value was recorded by the control red cv cocoyam plants, 0.06±0.01 mg g^{-1} FW after 6months of growth (Fig 5B)

Table 1. Physical analyses of soil before planting and after harvest

Physical properties (%)	**Farm before planting**	**Treatments plots after harvest**				
		Control	Poultry manure (20t ha^{-1})	Poultry manure (30t ha^{-1})	NPK (120kg ha^{-1})	NPK (150kg ha^{-1})
Moisture content	3.47	3.07	4.38	5.39	3.72	3.77
Sand	29.3	28.3	29.8	29.1	29.2	28.8
Silt	35.6	35.5	34.8	35.3	35.6	35.4
Clay	35.0	36.1	35.3	35.5	35.1	35.7
Textural class	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam

Table 2. Chemical analyses of soil before planting and after harvest

Chemical properties	Farm before planting	Treatments plots after harvest				
		Control	Poultry manure (20t.ha^{-1})	Poultry manure (30t ha^{-1})	NPK (120kg ha^{-1})	NPK (150kg ha^{-1})
pH in Water	5.6	5.5	6.6	6.9	5.6	5.4
pH in KCl	4.4	4.2	6.4	6.8	4.7	4.4
Organic matter (g/kg)	23.44	21.75	48.31	55.85	23.41	21.49
Total N (g/kg)	1.1	1.1	1.4	1.7	1.5	1.6
Total C (g/kg)	13.44	12.61	28.02	32.40	13.73	12.26
Available P (mg/kg)	4.57	3.91	57.84	94.04	113.5	129.9
Exchangeable Ca (cmol/kg)	1.6	1.62	1.96	2.39	1.91	1.99
Exchangeable Mg (cmol/kg)	0.75	0.69	0.81	1.03	0.75	0.81
Exchangeable K (cmol/kg)	0.08	0.09	0.14	0.29	0.19	0.23
Exchangeable Na (cmol/kg)	0.10	0.10	0.49	0.53	0.21	0.31
Cation exchange capacity (cmol/kg)	5.0	5.0	6.0	6.0	5.6	5.6

Table 3. Growth parameters of white cv *X. sagittifolium* plants

Treatment	Average plant height (cm)				Average number of leaves				Average leaf area (m^2)			
	2map	4map	6map	8map	2map	4map	6map	8map	2map	4map	6map	8map
Control	35^{e}	42^{e}	55^{e}	25^{e}	2^{a}	3^{ab}	2^{c}	1^{a}	0.031^{d}	0.021^{e}	0.025^{e}	0.002^{e}
PM1	70^{b}	73.7^{b}	77^{b}	55^{b}	3^{a}	4^{ab}	5^{ab}	2^{a}	0.042^{b}	0.053^{b}	0.072^{b}	0.021^{d}
PM2	80^{a}	86^{a}	86^{a}	62^{a}	4^{a}	5^{a}	6^{a}	3^{a}	0.051^{a}	0.067^{a}	0.088^{a}	0.031^{a}
NPK1	40^{d}	47.7^{d}	60^{d}	39^{d}	2^{a}	2^{b}	3^{bc}	1^{a}	0.033^{d}	0.042^{d}	0.056^{d}	0.025^{c}
NPK2	50^{c}	60^{c}	70^{c}	45^{c}	4^{a}	4^{a}	3^{bc}	1^{a}	0.039^{c}	0.048^{c}	0.061^{c}	0.028^{b}
Significance	*	*	*	*	NS	*	*	NS	*	*	*	*

KEY: map= months after planting, PM1= Poultry manure (20t ha^{-1}), PM2= Poultry manure (30t ha^{-1}), NPK1= NPK fertilizer (120Kg ha^{-1}), NPK2=NPK fertilizer (150kg ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

Table 4. Growth parameters of red cv *X. sagittifolium* plants

Treatment	Average plant height (cm)				Average number of leaves				Average leaf area (m^2)			
	2map	4map	6map	8map	2map	4map	6map	8map	2map	4map	6map	8map
Control	23^{d}	41^{e}	50^{e}	40^{e}	2^{a}	3^{a}	4^{a}	1.5^{a}	0.020^{e}	0.031^{c}	0.041^{c}	0.018^{c}
PM1	29^{b}	51^{b}	60^{b}	38^{c}	3^{a}	3^{a}	5^{a}	2^{a}	0.027^{b}	0.043^{b}	0.065^{b}	0.021^{b}
PM2	35^{a}	62^{a}	77^{a}	59^{a}	4^{a}	4.3^{a}	5^{a}	3^{a}	0.034^{a}	0.054^{a}	0.069^{a}	0.031^{a}
NPK1	26^{c}	46^{d}	55^{d}	38^{c}	2^{a}	2.7^{b}	4^{a}	1.3^{a}	0.022^{c}	0.028^{d}	0.041^{d}	0.011^{d}
NPK2	30^{b}	48^{c}	58^{c}	51^{b}	3^{a}	3.3^{a}	4.5^{a}	1.6^{a}	0.025^{b}	0.041^{c}	0.058^{c}	0.013^{d}
Significance	*	*	*	*	NS	NS	NS	NS	*	*	*	*

KEY: map= months after planting, PM1= Poultry manure (20t ha^{-1}), PM2= Poultry manure (30t ha^{-1}), NPK1= NPK fertilizer (120Kg ha^{-1}), NPK2=NPK fertilizer (150kg ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability .

Table 5. Yield parameters of white cv *X. sagittifolium* plants.

Yield Parameters	TREATMENTS					Significance
	Control	PM1	PM2	NPK1	NPK2	
Tuber number plant^{-}	2^{c}	4^{bc}	8^{a}	3^{c}	6^{ab}	*
Tuber Weight (g)	20^{e}	100^{c}	250^{a}	80^{d}	230^{b}	*
Tuber Length (cm)	6^{c}	10^{b}	14^{a}	7^{c}	12^{ab}	*
Tuber Girth (cm)	10.5^{c}	13.5^{b}	18.5^{a}	11.5^{bc}	17^{a}	*

KEY: PM1= Poultry manure (20t.ha^{-1}), PM2= Poultry manure (30t.ha^{-1}), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

Table 6. Yield parameters of red cv *X. sagittifolium* plants

	TREATMENTS					
Yield Parameters	**Control**	**PM1**	**PM2**	**NPK1**	**NPK2**	**Significance**
Tuber number plant^{-}	2.3 ^{c}	4^{b}	5.7^{a}	2.7^{bc}	3.6^{bc}	*
Tuber Weight (g)	22^{e}	52^{c}	124.7^{a}	42^{d}	94^{b}	*
Tuber Length (cm)	3^{b}	5^{b}	8^{a}	5^{b}	8^{a}	*
Tuber Girth (cm)	6^{c}	7^{b}	14.5^{a}	8^{b}	13^{a}	*

KEY: PM1= Poultry manure (20t ha^{-1}), PM2= Poultry manure (30t ha^{-1}), NPK1= NPK fertilizer (120Kg ha^{-1}), NPK2=NPK fertilizer (150kg ha^{-1}), NS= Not Significant, * = Significant at 5% level of probability.

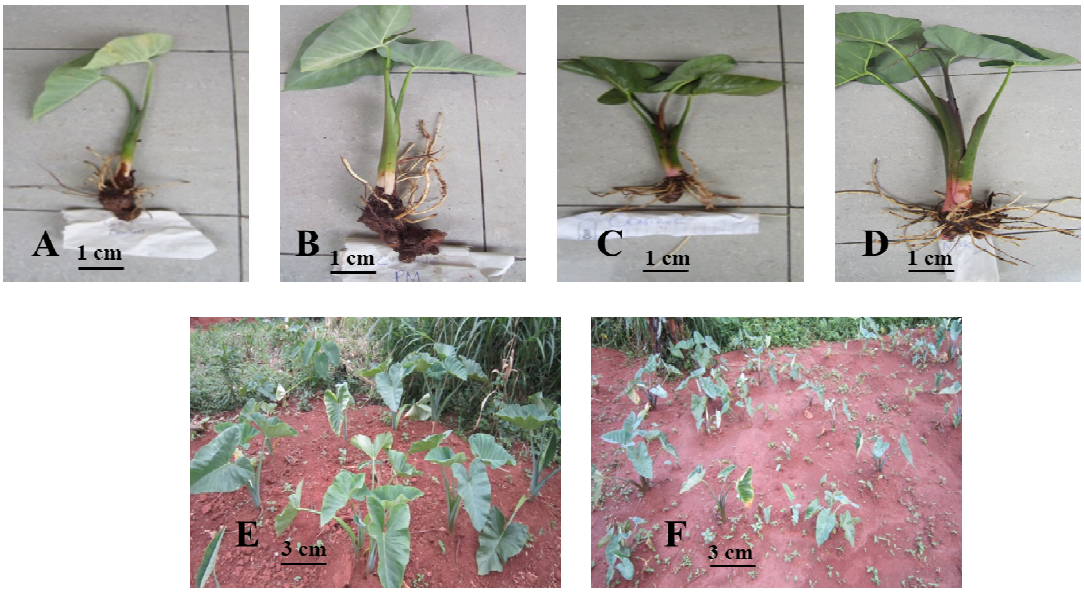

Fig.1. Aspect of cocoyam plant treatments after 6 months of growth: white cv control (A); white cv poultry manure (30tons/ha) (B); red cv control (C); red cv poultry manure (30 tons/ha) (D); white cv plot poultry manure (30tons/ha) (E); red cv plot poultry manure (30tons/ha)(F).

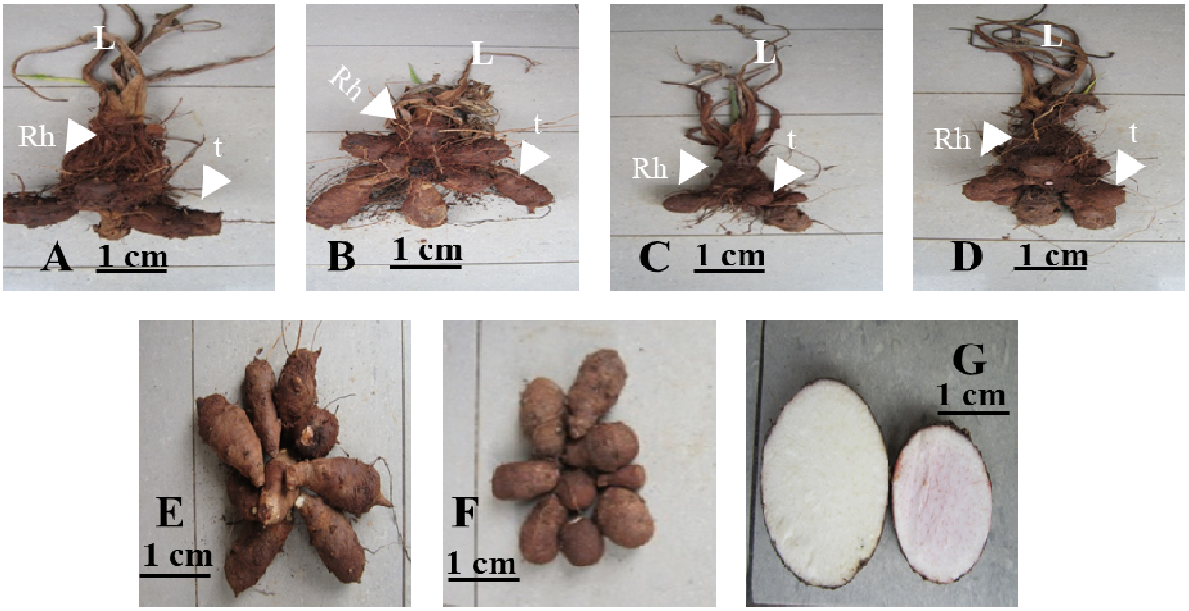

Fig.2. Aspect of cocoyam plant treatments at harvest after 9 months: white cv control (A); white cv poultry manure (30tons/ha) (B); red cv control (C); red cv poultry manure (30 tons/ha) (D); white cv tubers poultry manure (30tons/ha) (E); red cv tubers poultry manure (30tons/ha) (F); girth difference of white and red cvs (G). Dead leaves (L), Rhizomes (Rh) and tubers (t),

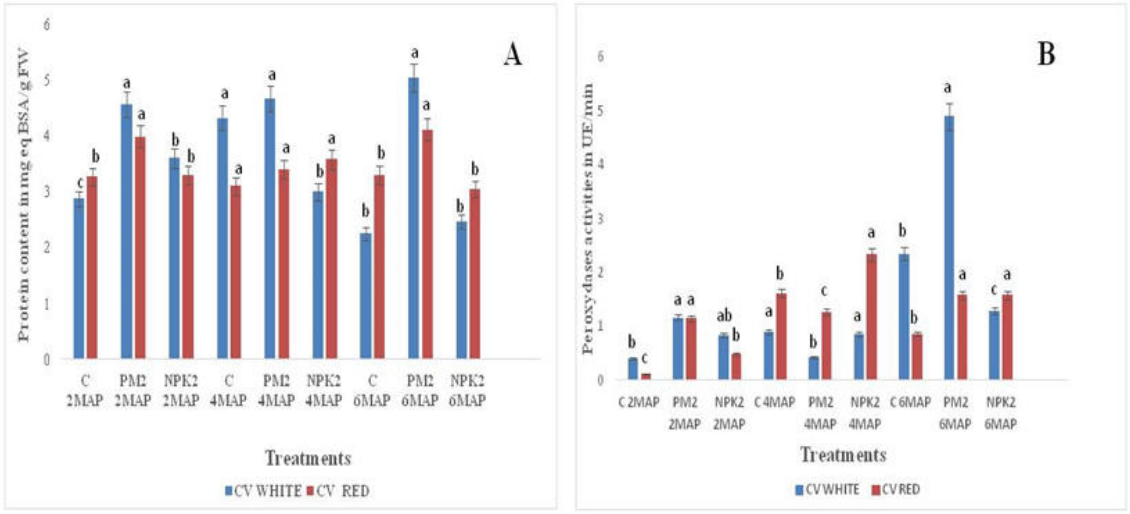

Fig.3. Protein content (mg eqBSA/FW) (A) and Peroxidase activities (UE/min) (B) in white and red cv cocoyam leaves during growth under different treatments. KEY: PM1= Poultry manure (20tons.ha^{-1}), PM2= Poultry manure (30tons/ha), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), MAP= months after planting.

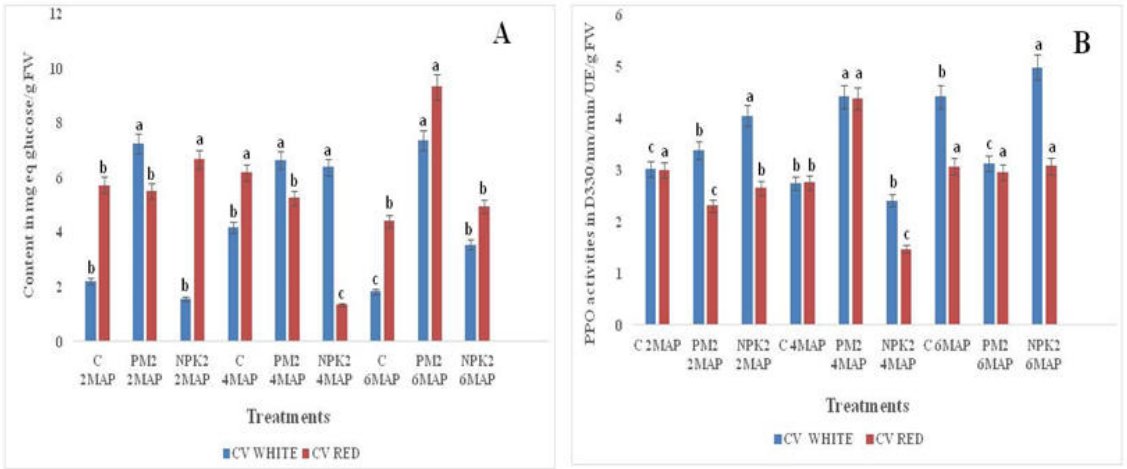

Fig.4. Glucanase activities (mg eq glucose.g^{-1} FW) (A) and Polyphenol oxidase activities (D330/nm/min/UE/g FW) (B) in white and red cv cocoyam leaves during growth under different treatments. KEY: PM1= Poultry manure (20tons.ha^{-1}), PM2= Poultry manure (30tons/ha), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), MAP= months after planting.

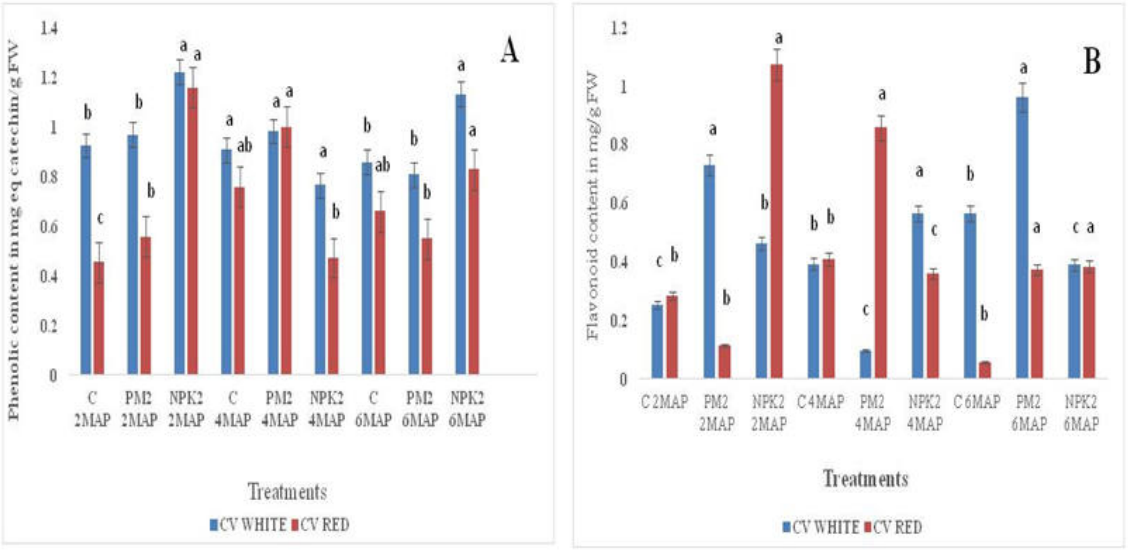

Fig.5. Phenolic content (mg eq catechin/g FW) (A) and in Flavonoid content (mg.g^{-1} FW) (B) in white and red cv cocoyam leaves during growth under different treatments. KEY: PM1= Poultry manure (20tons.ha^{-1}), PM2= Poultry manure (30tons/ha), NPK1= NPK fertilizer (120Kg.ha^{-1}), NPK2=NPK fertilizer (150kg.ha^{-1}), MAP= months after planting.

4. DISCUSSION

Effects of poultry manure and NPK fertilizer treatments on growth and yield parameters of the white and red cultivars of cocoyam (*Xanthosoma sagittifolium* L. Schott) minituber seeds were assessed. The growth and yield performance for the white cultivar cocoyam plants were significantly higher than those for the red cultivar cocoyam plants. These results are concordant with those obtained by Nzietchueng, 1985 who evaluated the production problems faced by different cocoyam (*Xanthosoma*) cultivars and showed that the white cultivar was more productive than the red cultivar. As observed from the results, it was evidenced that poultry manure (30 t ha^{-1}) treatments significantly produced the highest means of traits assessed, while the control treatments of no poultry manure or no NPK fertilizer applied significantly produced the lowest means of the same traits over a 9 month growth and yield period in both cultivars.150 kg ha^{-1} NPK fertilizer treatments expressed the most significant means of traits among all the NPK fertilizer treatments in both cultivars. Similar observations were earlier reported by Hamma *et al.,* 2014, when they evaluated the performance of cocoyam (*Colocasia esculentus.L*) under the influence of organic and inorganic manure in Samaru, Zaria, Nigeria. They observed that poultry manure (10 t ha^{-1}) produced the most significant means of growth and yield parameters among treatments of no manure (control), goat manure (10 t ha^{-1}) and cow manure (10 t ha^{-1}). They also showed that 150 kg ha^{-1} NPK fertilizer treatments produced the most significant means of growth and yield traits among no NPK fertilizer (control), 90 kg NPK ha^{-1} and120 kg NPK ha^{-1} fertilizer treatments.

The fact that these results depicted better crop performance in all parameters measured with poultry manure (30t ha^{-1}) than in all NPK fertilizer treatments for both white cv and red cv could be attributed to the fact that poultry manure treatments had a more favourable influence on soil pH, and soil organic content by increasing them and recorded higher cation exchange capacity values than all other treatments. Similar results were obtained by Gülsüm *et al.,* 2019 who showed that poutry manure applications improved soil organic matter content, exchangeable cations, cation exchange capacity, and percent base saturation thereby enhancing yield of sweet basil (*Ocimum basilicum* L.).Therefore poultry manure treatment plots retained more nutrients in the soil than the other treatments and slowly released these nutrients to the plants with reduced leaching losses of nutrients throughout the growth period Uwah *et al.,* 2011. The chemical composition of the different treatment plots after harvest (Table2.) showed that those treated with poultry manure (30 t ha^{-1}) had a higher N, K and organic carbon content which could also account for better growth and yield performance. These results are similar to those obtained by Uwah *et al.,* 2011 , who evaluated the effect of organic and mineral fertilizers on growth and yield of taro (*Colocasia esculenta* (L.) Schott and showed that when the N,P,K and Ca contents of poultry manure increased on a site, it led to superior crop performance. Concordant results have also been obtained by Karamat *et al.,* 2019 who showed that poultry litter had a significant increase in grain yield and N,P,K uptake by corn (*Zea mays*).

Protein content was most significant in white cv plants treated with poultry manure (30 t ha^{-1}) after 6 months of growth. These results are contrary to those obtained by Borgmann *et al.,* 1994 who showed that the

total protein content decreased during the maturation of microtubers in irish potatoes. Since proteins play an important role in the growth and repair of plant cells, this could suggest a positive correlation between protein content and the different growth parameters assessed during growth of plants treated with Poultry manure (30t ha^{-1}). The control and NPK2 treatments of both cultivars recorded a decrease in protein content between 4months and 6months after planting (Fig.3), corroborating results obtained by Djeuani, 2017 obtained a decrease in leaf protein content over time especially during maturation of cocoyam minitubers.

Biotic or abiotic stress may account for the significant increase in peroxidise activity noticed in the white cv plants treated with Poultry manure (30 $t.ha^{-1}$) and the during the first 6 months of growth. These results are similar with those obtained by Djocgoue,1998 ; Baaziz *et al.,* 2006;Mas & Heng, 2019, who showed that generally, in case of a wound, microbial infection or any other unfavourable condition, new isoperoxidases appear.

Our results also illustrate very significant levels of glucanase activities in both cultivars treated with poultry manure (30 t ha^{-1}) after 6 months of growth. Hereby suggesting roles of glucanases in plant stress relief and agreeing with Vaiyapuri *et al.,* 2012 who evidenced that β 1,3 glucanases play key roles in cell division, impeding cell to cell virus movements in plants by regulating callose turnover at plasmodesmata, and withstanding abiotic stress and Xiaohui *et al.,* 2019 who revealed the involvement of gluanases in fungal growth inhibition mechanisms by the rhizobacteria (*Paenibacillus jamilae* HS-26).

Polyphenol oxidases(PPOs) catalyse the oxidation of phenolic compounds into highly reactive quinones. Polyphenol oxidase activities were significant in white cv plants after 6 months of growth and in red cv plants after 4 months of growth both treated with NPK_2 (150 kg ha^{-1}). These results agree with Steffens *et al.,* 1994 who suggested that *in vivo* polyphenol oxidase activity can be associated to senescing, wounding or damage to plant tissues in which cellular compartmentalization is lost. This is justified by the fact that classically polyphenol oxidases and their potential phenolic substrates are physically separated from one another in plants. Polyphenol oxidases are found in chloroplasts while phenolic compounds are found primarily in the vacuole and cell wall (Vaughn *et al.,* 1998). Similarly, Ioannis *et al.,* 2019 had results which indicated that PPOs could accept flavonoids as their natural substrates and therefore might participate in the synthetic pathways of secondary metabolites.

At 2 months after planting both cultivars treated with NPK_2 (150 kg ha^{-1}) recorded the most significant phenolic content values. NPK_2 may have increased soil salinity, leading to the formation of large amounts of oxygen free radicals (O_2•-) in peroxisomes. Accumulation of ROS results in “oxidative stress”. ROS initiate free radical reactions that lead to oxidation of proteins, lipids and nucleic acids impairing their functions and causing cell death. This therefore justifies an increase in phenolic contents which form an antioxidant system developed by plants to counter “oxidative stress” by capturing ROS (Gill & Tutejn, 2010;Sharma *et al.,* 2012). Mas & Heng, 2019 also depicted that total phenolic content in the extracts of *Pereskia bleo* leaves significantly influenced antioxidant and antimicrobial activities.

Significant flavonoid content values were recorded 6 months after planting in white cv plants and 4 months after planting in red cv plants both treated with poultry manure (30 t/ha^{-1}). Flavonoids participate in plant protection against biotic (herbivores, microorganisms) and abiotic stresses (UV radiation, heat), and due to their antioxidative properties, they also maintain a redox state in cells. The antioxidative activity of flavonoids is connected with the structure of the molecule: the presence of conjugated double bonds and the occurrence of functional groups in the rings (Amić, et al., 2003;Seyoum *et al.,* 2006;Ireneusz *et al.,* 2018;Wang *et al.,* 2019). Flavonoids reduce the production of and quench reactive oxygen species (ROS) through: suppression of singlet oxygen; inhibition of enzymes that generate ROS (cyclooxygenase, lipoxygenase, monooxygenase, xanthine oxidase); chelating ions of transition metals, which may catalyze ROS production; quenching cascades of free-radical reactions in lipid peroxidation;“re-cycling” of other antioxidants (Rice-Evans *et al.,* 1996;Cotelle *et al.,* 1996;Arora *et al.,* 2000).

5. Conclusion

The objective of this pioneering field trial of *Xanthosoma sagittifolium* (white and red cultivars) minitubers as seeds was to evaluate their performance in response to different treatments of poultry manure and NPK fertilizers. Our results show that for all treatments, the white cultivar *X. sagittifolium* minitubers depicted better growth and yield parameters than the red cultivar minitubers. Poultry manure (30t ha^{-1}) stimulated the most significant responses in terms of better growth and yield among all treatments in both cultivars, suggesting that poultry manure offers a higher nutrient content and a more favourable pH to the soil than the other treatments. Both poultry manure (30 t ha^{-1}) and NPK (150kg ha^{-1}) treatments in both cultivars significantly influenced total soluble protein content, peroxidase activity, glucanase activity, polyphenoloxidase activity, phenolic contents and flavonoid content in the leaves during growth. These secondary metabolites play key roles in controlling abiotic and biotic stress thereby enhancing growth and development in plants. This work serves as an initial step in the improvement of cocoyam (*Xanthosoma sagittifolium*) production in Cameroon using cocoyam minitubers

as seeds.

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Conflicts of Interests
The authors have not declared any conflict of interest

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