ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Journal of Agronomy

ISSN 1812-5379
DOI: 10.3923/ja.2018.147.153

Research Article
Effect of Fungicide Application on Some Nutritional and Anti-nutritional Composition of *Colocassia esculenta* (Cocoyam)

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Abstract

Background and Objective: Staple food remain the major source of foods in Nigeria and West Africa. However, due to challenge of diseases, there have been a lot of setbacks in its farming and processing. This study investigated if application of fungicide affects nutritional composition of *Colocassia esculenta* (cocoyam). **Materials and Methods:** Fungicide was applied foliarly and farming season lasted for 8 months. All farming conditions for proper growth of the cocoyam were achieved. At the end of farming, amino acid analysis, phytochemicals and nutrient composition were determined using standard protocols. **Results:** Result obtained showed reduced phytocompounds in the control group when compared to the test group treated with the fungicide. Alkaloid (1.72±0.55, 3.07±0.18), flavonoid (3.96±0.95, 4.31±0.03), Tannins (0.65±0.22, 0.17±0.05), saponin (1.81±0.01, 3.00±0.05), Glycoside (5.26±0.10, 9.23±0.20), oxalate (0.58±0.03, 1.98±0.16) and phytate (0.31±0.03, 1.51±0.10) in control and test group respectively. Result for proximate composition showed reduced crude fibre (3.43±0.24, 1.12±0.07), starch (20.33±0.32, 15.50±0.57), amylose (6.72±0.16, 4.97±0.09) and lipid content (1.85±0.28, 0.96±0.09), reduced vitamin content, reduced amino acid content and increased mineral contents in the group applied fungicide when compared to control. **Conclusion:** It could be concluded from the results obtained this study that the fungicide used, affected the nutritional and anti-nutrient composition of cocoyam.

Key words: Fungicide, *Colocassia esculenta*, amino acid analysis, phytocompounds, anti-nutrient

Citation: Thecla Nchedo Mbah, Adanma Chiemeziem Obike, Emmanuel Uchenna Ejiofor, Gavin Chibundu Ikechukwu, Gabriel Chinedu Mbah and Yusuf Ndukaku Omeh, 2018. Effect of fungicide application on some nutritional and anti-nutritional composition of *Colocassia esculenta* (cocoyam). J. Agron., 17: 147-153.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cocoyam (*Colocassia esculenta*) is a major contributor to the human diet in Africa especially in Nigeria and West Africa[1]. It has been ranked one of the majorly consumed tuber on earth[2]. The most important parts of the plant are its starchy corms and cormels, used as a subsistence staple, providing a cheap source of carbohydrates. Cocoyam serves as a source of income for many families in the tropics and subtropics[3]. Cocoyam is known to contain considerable amount of protein, starch, vitamins and minerals, while carbohydrates occur at the highest[4]. The major nutrient obtained from it consumption is carbohydrate, yielding energy[5]. The tuber is poor in proteins and concentration of Sulphur contain amino acids are limiting. One major essential mineral nutrient in cocoyam is Potassium[6] also rich in Iron, Zinc, Calcium[7]. Njoku and Ohia[8] reported that cocoyam is a good source of sodium, potassium, magnesium and calcium, whose salts are regulators of the acid-base balance of the body.

One problem affecting cocoyam farming is the Taro Leaf Blight Disease. The disease is caused by the foliar pathogen *Phytophthora colocasiae*, affecting the petioles and corms of its host. Usually, at initial stage of the disease, small, dark brown flecks or light brown spots are spotted on the leaf surface especially the point of water accumulation on leaves. As disease progresses, spots are enlarged till the leaves are fully damaged[9]. The disease is known to negatively affect the performance of the affected plant, thus reducing plant yield and nutritional composition[10]. The management of the disease involves cultural practices which may include field sanitation, chemical management (which looks at use of fungicide and pesticides) and genetic resistance[9]. However, chemical method has been widely used in the management of the disease, considering it cheap cost and high protective ability. The use of chemicals like fungicide has been reported to control Taro Leaf Blight[11]. However, the effect of chemical application to combat the disease on the nutritional composition of the disease have not been fully studied, Hence this study was conducted to evaluate whether fungicide application could positively or negatively affect the starch quality of cocoyam, as there was paucity of information on the effect of fungicide application on other nutritional contents (proximate and minerals) of cocoyam.

MATERIALS AND METHODS

Time, duration and location of study: The study was conducted between the months of January, 2016 to November, 2016. The location of the study was the experimental site which is the Cocoyam Farm of the National Root Crop Research Institute, Umudike. The whole study through planting and data analysis lasted for 11 months.

Plant material sampling: The cocoyam varieties used for the study were NCE011 and NCE012 variants. Clean and healthy cocoyam samples were collected from Gocin Barn of National Root Crops Research Institute, Umudike. The cocoyam was authenticated by Dr. Garuba Omosun, a taxonomist at the Department of Plant Science and Biotechnoloy, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, as *Colocassia esculenta*.

Materials: Fungicide and fertilizer used were obtained from an agro-chemical store in Umuahia, Abia State. Other chemicals such as HCl, methanol, acetic acid, picric acid, sodium chloride, sodium benzoate, sodium nitrite, magnesium chloride, phosphate tablets, sulphanilic acid were Sigma Aldrich Lagos distributor.

Experimental design: The experimental site which is the Cocoyam Farm of the National Root Crop Research Institute, Umudike, was divided into two groups. The distance between the two groups was 100 m. To one group, termed the control, NPK 15:15:15 fertilizer was applied six weeks after planting of the cocoyam. The matured cocoyam's were harvested 8 months after planting. To the second group termed the test group, NPK 15:15:15 fertilizer was applied six weeks after planting of the cocoyam. The fungicide (Ridomil) was applied every 4 weeks after planting at a rate of 50 g of sachet into knapsack of 5 L of water. The matured cocoyam's were harvested 8 months after planting. Experimental plot was Randomized Complete Block Design (RCBD) replicated three times. All other farming conditions for proper growth of cocoyam's were strictly adhered to such as weeding, watering, use of traps to catch rodents and use of scary objects against birds.

Phytochemical content determination: The following methods were used for Alkaloids determination[12], Saponins determination[12], Tannins determination[13], Phenol determination[13], Cyanogenic glycosides determination[14]. Phytic acid determination[15].

Proximate contents determination: The Association of Official Analytical Chemist (AOAC)[16] methods were used to determine the proximate compositions viz: moisture content, ash content, crude fibre content, crude protein content, fat content, carbohydrate content.

Determination of vitamins: Following vitamins were determined Retinol[17], Thiamine[17], riboflavin[17], niacin[17] determination[17] and ascorbic acid[18].

Determination of minerals: The mineral content of the sample was determined by the dry ash acid extraction method described by James[19]. A measured weight of the sample (2 g) was burnt to ashes (as in ash determination) in a muffle furnace at 550°C. The resulting ash was dissolved in 10 mL of 2 M HCl solution and diluted to 90 mL of distilled water in a volumetric flask and filtered. The filtrate was used on the mineral analysis. In minerals calcium and magnesium[19], phosphorus[19] (James[19]), sodium and potassium[17] content were determined.

Amino acid concentration: This was achieved using the amino acid analyzer as described by Spackman *et al.*[20] using Technicon Sequential Multi-Sample Amino Acid Analyser.

Statistical analysis: Statistical analysis was carried out using SPSS Ver. 22. Data obtained were subjected to Student-T-test for significant difference between means. Probability value was set at 95% confidence level for all studied parameters.

RESULTS

From the result presented in Table 1, alkaloid, flavonoids, saponins, glycosides, oxalate and phytate concentration was significantly ($p<0.05$) higher in the test group when compared to the control group. Result for phenols and tannins was shown to be significantly ($p<0.05$) lower in the test group when compared to the control group.

Result of proximate composition was presented above in Table 2. Result for dry matter and ash was significantly ($p<0.05$) lower in the control group when compared to the test group. Moisture content, crude protein, crude lipid and crude fibre was significantly ($p<0.05$) lower in the test group when compared to the control group. Result for carbohydrate and energy value showed no significant difference.

Result presented in Table 3 showed that starch and amylose content was significantly ($p<0.05$) lower in the test group when compared to the control group. Amylopectin content was significantly ($p<0.05$) higher in the test group when compared to the control group.

Result for vitamin and reducing sugar was present in Table 4. Vitamin A, B, C and reducing sugar was significantly ($p<0.05$) lower in the test group when compared to the control group.

Result for mineral estimation was presented in Table 5 above. Calcium concentration was significantly ($p<0.05$) lower in the test group when compared to the control group. Result for Sodium, Potassium, Magnesium, Zinc and Iron was significantly lower in the control when compared to the test group.

Result for functional analysis of cocoyam flour was presented in Table 6 above. Result for bulk density, wettability and gelation time was significantly ($p<0.05$) lower in the test when compared to control. Water absorption capacity and swelling index was significantly ($p<0.05$) lower in the control when compared to the test group. Result for oil absorption capacity showed no significant ($p<0.05$) difference.

Result for amino acid composition was presented above in Table 7. Eighteen amino acids were detected and analyzed. Leucine concentration was significantly ($p<0.05$) lower in the control when compared to the test group. Lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, arginine, tyrosine, cysteine, alanine, glutamate, glycine, aspartate was significantly ($p<0.05$) lower in the test when compared to the control group. Proline, histidine, threonine and serine showed no significant ($p<0.05$) difference.

Table 1: Phytochemical screening of leaves

	ALK	FLAV	TANIN	SAP	PHEN	GLY	OXA	PHY
Phytochemical	----------	----------	----------	----------	($mg\ g^{-1}$)	----------	----------	----------
Control	1.72±0.55[a]	3.96±0.95[a]	0.65±0.22	1.81±0.01[a]	0.06±0.01	5.26±0.10[a]	0.58±0.03[a]	0.31±0.03[a]
Test	3.07±0.18	4.31±0.03	0.17±0.05[b]	3.00±0.05	0.01±0.00[b]	9.23±0.20	1.98±0.16	1.51±0.10

Values are reported as Mean±SD. [a]Significantly ($p<0.05$) lower than the test group in the same column, [b]Significantly ($p<0.05$) lower than the control group in the same column, Alk: Alkaloids, Flav: Flavonoids, Tan: Tannins, Sap: Saponins, Phen: Phenols, Gly: Glucosides, Oxa: Oxalate, Phy: Phytate

Table 2: Proximate composition of cocoyam

Group	MC (%)	DM (%)	CP (%)	CL (%)	ASH (%)	CHO (%)	Energy (kcal)	CF (%)
Control	75.30±1.83	24.70±0.18[a]	2.27±0.18	1.85±0.28	2.78±0.17[a]	64.92±1.47	275.37±2.71	3.43±0.24
Test	71.25±0.81[b]	28.65±0.81	1.78±0.10[b]	0.96±0.09[b]	3.08±0.09	64.38±0.73	273.37±3.46	1.12±0.07[b]

Values are reported as Mean±SD. [a]Significantly ($p<0.05$) lower than the test group in the same column, [b]Significantly ($p<0.05$) lower than the control group in the same column. MC: Moisture content, DM: Dry matter, CP: Crude protein, CL: Crude lipids, CHO: Carbohydrates, CF: Crude fibre

DISCUSSION

The present study investigated the possible effects of fungicide application (Ridomil) on nutritional properties of cocoyam. Result for phytochemical study, showed a significant ($p<0.05$) increase in all studied phytochemical except phenol for the test when compared to the control group. One major site of attack of the fungi that caused the Taro disease is the leaf of cocoyam, where it leaves black or brownish spots on the leaves[21]. Singh *et al.*[22] reported that the lesions on the leaves were the result of oomycetes draining nutrients out of the leaves through haustoria to create white powdery rings of sporangia. Plants are known to contain phyto compounds with therapeutic activities[23]. Generally, leaves of plant are good sources of these phyto compounds. During taro disease of cocoyam, the leaves of the plants are not spared as the organism generate spores on the leaves, which can eat up the leaves, forming black or brownish spots on the leaves[21].

Result for phenols was significantly ($p<0.05$) higher in the control group when compared to the test group (Fungicide treated group). This can be attributed to the generation of free radicals by the fungicide[24]. This agrees with the findings of Marques *et al.*[25], who reported an increased level of malondialdehyde (MDA) in fungicide treated wheat when compared to the control. MDA is a widely accepted biomarker for lipid peroxidation and oxidative stress in plants and animals that can be arising from toxic compounds. Phenols however are well known for the role they play in the fight against free radical[26]. Since the fungicide works by generation of free radicals to kill the organism, some of the free radicals which find their way to the leaves may have generated oxidative stress that led to the depletion of phenol in the test group.

The depletion or degradation of plant phytochemicals can be attributed to the harmful effect of the Taro disease as seen in the control group without fungicide treatment. More so, on physical observation of the leaves, more spots were discovered on leaves without treatment when compared to leaves of plant treated, indicating the fungicide could inhibit growth of fungal spores.

Result for proximate composition, showed a significant ($p<0.05$) decrease in the moisture content of the test group when compared to the control group. The reduced moisture content can be one of the mechanism in which the fungicide uses to protect the crop from fungal growth. Water is well known to promote spoilage of tubers and a strong requirement for microorganisms to survive[27]. Result for dry matter was significantly ($p<0.05$) high in control when compared to the test group. This corroborated the result obtained for moisture content. Generally, high moisture content relates to low dry matter. Result for crude protein, lipid and fibre was significantly ($p<0.05$) high in control group when compared to the test group. This indicates that the fungicide had an adverse effect on the fibre, lipid and protein content of the cocoyam. This can be attributed to the petrochemicals present in the fungicides that can damage lipid and amino acid content of plants[28]. Swoboda and Pedersen[29] however reported that fungicide application has no effect on yield and protein content in soy beans. Ash

Table 3: Starch, amylose and amylopectin content of cocoyam

Starch	Starch (%)	Amylose (%)	Amylopectin (%)
Control	20.33±0.32	6.72±0.16	93.17±0.14[a]
Test	15.50±0.57[b]	4.97±0.09[b]	95.02±0.09

Values are reported as Mean±SD. [a]Significantly ($p<0.05$) lower than the test group in the same column, [b]Significantly ($p<0.05$) lower than the control group in the same column

Table 4: Vitamin content of cocoyam

	Vitamin A	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin C	Red sugar
Group	(mg/100 g)					
Control	74.50±1.73	0.89±0.00	0.19±0.00	0.41±0.01	5.68±0.08	2.17±0.30
Test	61.50±0.57[b]	0.02±0.00[b]	0.01±0.00[b]	0.28±0.00[b]	4.21±0.11[b]	0.88±0.04[b]

Values are reported as Mean±SD. [b]Significantly ($p<0.05$) lower than the control group in the same column

Table 5: Mineral content of cocoyam

	CA	NA	K	MG	ZN	FE
Group	(mg/100 g)					
Control	42.05±0.31	68.18±3.35[a]	182.38±1.87[a]	52.21±0.56[a]	0.17±0.00[a]	3.41±0.25[a]
Test	30.07±1.13[b]	89.93±15.89	197.05±6.03	85.54±7.40	0.71±0.09	4.96±0.60

Values are reported as Mean±SD. [a]Significantly ($p<0.05$) lower than the test group in the same column, [b]Significantly ($p<0.05$) lower than the control group in the same column. Ca: Calcium, Na: Sodium, K: Potassium, Mg: Magnesium, Zn: Zinc, Fe: Iron

Table 6: Functional property of cocoyam flour

	Bulk density	WAC	OAC	Wettability	Swelling index	Gelation time
Group	(%)					
Control	0.69±0.00	2.49±0.02[a]	1.46±0.11	7.09±0.01	22.25±0.46[a]	88.37±1.30
Test	0.53±0.01[b]	2.96±0.07	1.46±0.12	6.08±0.07[b]	25.87±0.99	72.75±1.66[b]

Values are reported as Mean±SD of triplicate determination. [a]Significantly ($p<0.05$) lower than the test group in the same column, [b]Significantly ($p<0.05$) lower than the control group in the same column. WAC: Water absorption capacity, OAC: Oil absorption capacity

Table 7: Amino acid composition of cocoyam

Group	Control (g/100 g protein)	Test (g/100 g protein)
Leucine	3.32±0.05[a]	8.81±0.07
Lysine	2.66±0.03	1.81±0.02[b]
Isoleucine	2.76±0.02	2.51±0.02[b]
Phenylalanine	4.13±0.09	3.49±0.00[b]
Tryptophan	2.06±0.00	1.50±0.00[b]
Valine	3.62±0.03	3.00±0.00[b]
Methionine	1.17±0.03	0.88±0.02[b]
Proline	1.06±0.03	1.16±0.02
Arginine	4.13±0.04	3.80±0.02[b]
Tyrosine	2.76±0.04	2.04±0.04[b]
Histidine	2.02±0.02	2.03±0.04
Cysteine	1.14±0.02	0.68±0.02[b]
Alanine	1.96±0.02	1.67±0.03[b]
Glutamate	7.66±0.22	5.70±0.00[b]
Glycine	3.47±0.02	2.40±0.13[b]
Threonine	2.09±0.86	2.00±0.00
Serine	1.78±0.01	1.81±0.14
Aspartate	6.78±0.03	6.07±0.19[b]

Values are reported as Mean±SD. [a]Significantly ($p<0.05$) lower than the test group in the same row, [b]Significantly ($p<0.05$) lower than the control group in the same row

content was significantly ($p<0.05$) lower in the control group when compared to the test group. Ash content can serve as a predictor for mineral content of an organic material. The increase in ash content of the group applied fungicide can be attributed to the inorganic elements presents in the fungicide which are absorbed by soil after application of the fungicide and the inhibition of the fungi from degrading the cocoyam. Result for carbohydrate composition and energy value showed no significant ($p<0.05$) difference. This indicates that the fungicide may not have an adverse effect on the energy yield or value of cocoyam.

Result for starch and amylose composition was significantly ($p<0.05$) decreased in the test group compared to the control, while that of amylopectin was significantly ($p<0.05$) higher in the test group when compared to the control group. This clearly indicates that prolonged administration of the fungicide may affect starch yield of the cocoyam. Cocoyam's are known for its high starch content and are widely used as thickeners in cooking[30]. However, amylose and amylopectin makes up the component of starch, with amylopectin being responsible for the gel nature of starch[31]. The increase in amylopectin in the test group and increase in amylose composition in the control indicates that the fungicide may contain compounds that promote the activities of amylase.

Result for vitamins showed that vitamin A, B_1, B_2, B_3 and C concentration was significantly ($p<0.05$) higher in the test group when compared to the control group. The decrease in vitamin concentration in the group treated with the fungicide can be linked with the oxidative stress that may be caused by the compounds such as petrochemicals which are present in the fungicide. These compounds can alter redox balance in cell and since vitamins possess antioxidant activity, they can be used to mop up or degrade the free radicals generated by these compounds. Also, this reflected in the composition or quantity of reducing sugars. Reducing sugar was significantly ($p<0.05$) higher in the control when compared to the test group. Reducing sugars can act as a reducing agent because of it free aldehyde or ketone group. These reducing agents can act as reductants for oxidized molecules, especially antioxidants and may play a role in protecting the cells from damage from oxidants.

Result for mineral estimation corroborated result obtained for ash, since ash content can serve as indicator for mineral content of an organic material. Result for sodium, potassium, phosphorous, magnesium, zinc and iron was significantly ($p<0.05$) higher in the test group when compared to the control. The increase in these minerals can be attributed to the mineral content of the fungicide, which after application to the soil, may be absorbed by the plants. Also, one point of attack by the fungi, maybe the mineral content of the plant, which it may rely on for its own use. However, the fungicide played a role in inhibiting the growth of the fungi and thus may act by inhibiting the uptake of these minerals by the fungi. However, the fungicide did not improve calcium concentration in the test group as seen by the result of this study.

For the functional properties of the cocoyam, BD, WET and GT were significantly ($p<0.05$) lower in the test group when compared to the control group. However, WAC and SI was significantly ($p<0.05$) lower in the control when compared to the test group. The bulk density would normally be low considering the low moisture content of the treated group. Also, the water absorption capacity of the test will be high and the wettability low due to the reduced moisture content of the cocoyam treated with the fungicide. Reduction in GT (Gelation time) and increase in swelling index in the test group, can be linked to low reducing sugar in the test group. Also, the presence of low starch, will lead to an increase in swelling index and reduction in gelation time.

For amino acid analysis, threonine and serine showed no significant difference, indicating the fungicide did not alter their concentration. However, leucine was significantly ($p<0.05$) higher in the test group, when compared to the control group. Pyruvic acid is a major substrate for leucine biosynthesis in plants and the pathway involves six enzymes. The result of this study indicates that the fungicide may have the ability to enhance the enzymes of the pathway, or promote the increase formation of pyruvic acid, thus leading

to high leucine concentration. The fungicide may promote dark phase respiration which leads to the breakdown of stored starch, leading to formation of pyruvic acid. This also shows that one point of attack of the fungi could be dark respiration or inhibition of major enzymes of the plant. Also, the result of this study showed that the highest occurring amino acid in cocoyam is leucine, which is an essential amino acid. Aspartate is an amino acid that acts as precursor for four other amino acids which includes methionine, threonine, isoleucine and lysine. A reduction in aspartate concentration will literally lead to a reduction in the other four amino acids, as indicated in this study. All other studied amino acid in this study were significantly ($p<0.05$) lower in the test when compared to the control. This gives an indication that the fungicide may contain compounds that can inhibit the enzymes involved in the synthesis. Also, the free radicals generated from in tissues and cells of the plant once it is absorbed from the soil, can induce oxidative stress that can impair the synthesis of these amino acids, since it can alter or inhibiting the enzymes involved. Also, as free radicals are degraded or mopped up, the antioxidants are depleted and amino acids are strong requirement to synthesis them back. This may lead to a reduction in amino acid content of the test group as seen in this study. This agrees with the findings of Fromme *et al.*[32], who reported the oxidative stress damaged caused by fungicide application. Following the outcome of this study, the application of fungicide should be handled with utmost care. Number of times of application should be reduced, also, farmers who use fungicide to treat plants while the plants are in the field, should consider applying fungicide as a pre-treatment option before plant, or directly on the soil before planting. Although the damage done on cocoyam may not be toxic to human health, this study has shown that fungicide application affects plant nutritional status and may deposit toxic species in other plants (vegetables and fruits) which may be harmful when consumed by animals and humans.

CONCLUSION

It was concluded from this study that the fungicide negatively affects some nutritional composition of the plant and promoted the phytocompounds majorly in the plant leaves. It can also be deducted that the fungicide may have adverse effect on the enzymes of the plant. Thus, the application of fungicide should be targeted only to disease breakout or be employed with strict care, considering the amount of oxidative stress that may be generated from the fungicide application.

SIGNIFICANT STATEMENT

This study provides an insight on the possible effect of the application of fungicide on the nutritional composition of *Colocassia esculenta* (cocoyam) that can be of benefit to cocoyam farmers who treat their crops with fungicides. This study will help the researchers to uncover critical areas of the side effect of fungicide application and thus provide information and a new theory on how this happens and how it can be managed.

ACKNOWLEDGMENT

We are grateful to Mr. Ibeh Raymond, Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture, Umudike and Mr. Justice O. of the National Root Crops Research Institute also in Umudike for their technical assistance. We will not forget to acknowledge Ms. Chukwu C. Maureen, who participated in formatting manuscript according to journal standard. We also report that no grant was used or accessed for this study.

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