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Effects of Organic and Mineral Fertilizers on total Antioxidant, polyphenolic and carotenoid contents of Orange fleshed sweetpotato tubers

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Abstract

A field study was conducted to determine the effect of organic and mineral-based fertilizers on phytochemical contents in the tubers of an orange fleshed sweetpotato variety (TIB-440060). Treatments were arranged in a split plot design with three replicates. Tubers harvested have been studied for their total antioxidant contents (TAC), total polyphenolic contents (TPC) and total carotenoid contents (TCC) using Ferric Reducing Antioxidant Power (FRAP), Folin-Ciocalteu reagent (FCR) and a method described by McMurry respectively.

Organic fertilizer had a positive effect on the production of antioxidants, polyphenols and carotenoids whose contents increased significantly ($P < 0.001$) with the quantities of organic fertilizer. Increases about 19.61%, 34.41% and 12.82% due to annual (F1) inputs and increases about 15.94% and 15.46% and 12.17% respectively in total antioxidant, polyphenolic and carotenoid contents when these inputs of organic fertilizer are conducted biennially (F2).

The effect of different doses of mineral fertilizers indicated significant changes ($P < 0.001$) in accumulated antioxidant, polyphenolic and total carotenoids contents of TIB-440060 OFSP tubers.

Total Antioxidant, polyphenolic and carotenoid contents of TIB-440060 varied significantly ($P < 0.001$) with the combination of organo-mineral fertilization used. Combinations using a minimal doses of 15 kg/ha of nitrogen, 30 kg/ha phosphorus and 45 kg/ha of potassium associated with an annual or biennial contribution of 20 t/ha of organic fertilizer gave the highest total antioxidant, polyphenolic and carotenoid concentrations.

Keywords: OFSP extracts, phytochemical contents, polyphénols, antioxidants, carotenoids, organic fertilizer, mineral fertilizers, FRAP, FCR

1. INTRODUCTION

Increasingly, epidemiological studies indicate that consumption of a rich diet food from plant has a protective effect against cardiovascular disease and some forms of cancer (Savita *et al.,* 2010; Augustin *et al.,* 2011; Haiwen *et al.,* 2011). Although plants contain a wide range of compounds that could lead to their beneficial effects on health (proteins, amino acids, vitamins and fibre). Recent works focused on the role of antioxidant secondary metabolites of plants, in particular polyphenols and carotenoids in the prevention of diseases related to oxidative stress (Arts *et al.,* 2005).

Polyphenolic compounds vary considerably in their structure and their standings, but are characterized by the presence of at least one aromatic ring with one or more hydroxyl groups.

Carotenoids in foods are usually tetraterpenoids in C40 formed from eight isoprenoids C5 units interconnected to form a symmetrical molecule (Britton *et al.,* 1995).

Polyphenols and carotenoids are natural antioxidants with beneficial effects on human health (Osganian *et al.,* 2003; Meléndez-Martínez *et al.,* 2004; Tapiero *et al.,* 2004; Stahl *et al.,* 2005; Haiwen *et al.,* 2011). There is a growing interest to develop simple methods to increase the concentration of polyphenols in foods in order to strengthen their overall nutritional value (Parr *et al.,* 2000; Schreiner *et al.,* 2005; Schreiner *et al.,* 2006). Polyphenols and carotenoids are produced by plants throughout their development for various reasons: the defense against microorganisms, insects, and herbivores (Richard *et al.,* 1995; Crozier *et al.,* 2006); availability of nutrients (Richard *et al.,* 1995); exposure to ultraviolet radiation (Rozema *et al.,* 1997); and because of the allelopathic interactions (Mann *et al.,* 1987). However, because the reactions of plants to these stimuli are varied and not well understood, the use of these techniques to induce plants to produce secondary metabolites (and therefore potentially increase their nutritional value) is not common.

In particular, the availability of essential macronutrients in the growth of the plants has a significant potential to affect the accumulation of polyphenols (Parr *et al.,* 2000). Although it has been shown that levels of fertilization of nitrogen, phosphorus, potassium and calcium affect the production of secondary metabolites in plants (Osama *et al.,* 2011; Oloyede *et al.,* 2012; Bouchelaghem *et al.,* 2012; Nur *et al.,* 2012; R. Vignesh *et al.,* 2012), mineral nutrition has little or no effect on the production of polyphenols in some plants (Bímová *et al.,* 2008; Gupta-Elera *et al.,* 2012; Marilou *et al.,* 2012).

In this study, we explore how organic fertilizer consisting of well-decomposed livestock fertilizer and mineral fertilizers such as nitrogen (N), of phosphorus (P) and potassium (K) affect the production of total antioxidants, polyphenols and carotenoids from the bits of orange fleshed sweetpotato TIB-440060 variety (Koala et al., 2013). This work was undertaken to evaluate the effects of the accumulation of above phytochemicals of the studied orange-fleshed sweetpotato tubers (OFSP) harvested from soils treated with organic and/or mineral fertilizers.

2. MATERIALS AND METHODS

2.1 Material and methods

Chemicals and reagents: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Gallic acid, $Fe(III)(TPTZ)_2Cl_3$ (TPTZ = 2,4,6-tripyridyl-s-triazine), Ferric chloride were obtained from Sigma chemical Inc, USA. All other reagents and chemicals used were of analytical grade.

2.2 Sampling and sample preparation

The orange fleshed sweetpotato variety TIB-440060 was used the experiment established at the research station of Kamboinsé (INERA), 13 km from Ouagadougou in the centre of Burkina Faso. Two types of fertilizers were used: the organic fertilizer constituted by well-decomposed livestock manure and mineral fertilizers composed by nitrogen (N) fertilizer, phosphorus (P) and potassium (K). The experimental was conducted in a split plot design with in the main plots the organic fertilization in three (3) levels and the secondary plots containing the mineral fertilization in nine (9) levels (table 1). In total twenty-seven (27) combinations of organo-mineral fertilization have been established (table 2).

Drying of OFSP tubers: the sweetpotatoes storage root was peeled and left to dry in the laboratory for twenty days. To ensure that the samples were dried, the weight of each sample was measured every day until a constant weight was reached. The samples were then sealed in plastic soda-bag.

Extraction of plant material: 3 g of powder obtained by crushing the plant material were extracted three times by maceration with 10 mL of the following solvent systems: acetone-water-acetic acid (70: 29.5: 0.5 v/v) (Asami *et al.,* 2003) for TAC and TPC assays and acetone-hexane (50: 50, v/v) (Kowalski *et al.,* 2000) for TCC assay. Extracts were vigorously stirred for 30 minutes and then carefully filtered using filter paper Wathman N ° 1 and kept at 4 ° C protected from light in the refrigerator until the determination.

2.3 Determination of TAC using FRAP (Ferric Reducing Antioxidant Power) assay

In this method, a ferric salt, Fe (III) $(TPTZ)_2Cl_3$ (TPTZ = 2,4,6-tripyridyl-s-triazine) was used as an oxidant (Benzie *et al.,* 1996; Pulido *et al.,* 2000). It was prepared by mixing 1 mL (1 mL, 10 mM in 40 mM) TPTZ, 10 mL of a solution of sodium acetate buffer (pH = 3.6) and 1 mL of solution of Fe (III), H_2O (Pulido *et al.,* 2000). About 30 µL of distilled water was mixed to 20 µL of appropriately diluted extract and then, 200 µL of solution FRAP were added. Absorbances of the intense blue discoloration were measured at 593 nm using a microplate (spectrophotometer SAFAS, MP96) drive about 10 minutes after. A calibration curve was established using Trolox as reference of antioxidant. Results, determined from the calibration curve equation ($y = 28.67\ x + 0.066$; $R^2 = 0.999$), were expressed in mg of Trolox Equivalent (TE)/g dried weight. All measurements were performed in three replications.

2.4 Determination of TPC using Folin-Ciocalteu assay

Total phenolic phytochemical concentration was measured using the Folin-Ciocalteu method (Nihal *et al.,* 2007). Briefly, 60 µL of appropriate diluted samples and a standard solution of gallic acid (3,4,5-trihydroxybenzoic acid) were mixed to 60 µL of Folin-Ciocalteu's Reagent (FCR-1: 10 dilution) and left to stand for 8 min at room temperature to allow for the FCR to react completely with the oxidible substances or phenolates. 120 µL of Na_2CO_3 (7.5 % in water) were added to destroy the residual reagent. Absorbances were measured at 760 nm using a 96-well (glass vials 250 µL) microplate spectrophotometer (Microplate Autoreader MP96; SAFAS Instruments) after incubation for 30 min at 37 °C against distilled water as a blank. Total phenolic contents of the samples determined from the calibration curve equation ($y = 46.41\ x + 0.063$, $R^2 =$

0.998) were expressed in mg of gallic acid equivalents (GAE) per gram of dried weight. All measurements were performed in three replications.

2.5 Determination of total carotenoid contents (TCC)

Total carotenoid contents of OFSP extracts were evaluated according to the method described by McMurry (McMurry *et al.,* 2008; Jun *et al.,* 2011) slightly modified. A direct measurement of absorbance was made. After suitable dilution, the absorbances of extracts kept at room temperature and protected from light, were red at 455 nm. Total carotenoid contents were obtained by reporting absorbances of suitable diluted extracts on a curve ($y = 25.56\ x + 0.016$; $R^2 = 0.999$) established using β-carotene as standard. TCC were expressed in mg of β-carotene Equivalents (BCE)/g of dried weight. All measurements were performed in three replications.

2.6 Statistical analyses

All experiments were conducted in three replications. The results are expressed in mean ± SD. Analysis of variance (ANOVA) allowed to appreciate the differences between varieties for their antioxidant, polyphenolic and beta carotene contents using the statistical software Genstat, 14th Edition. The $p< 0.05$ values were considered statistically significant.

3. Results and discussion

Effect of organic fertilization: table 7 presents the effect of different doses of organic fertilization on total antioxidant, polyphenolic and carotenoid contents. TAC and TPC increased significantly ($P < 0.001$) (table 3) with the quantities of organic fertilizer. Indeed, on plots that received no supply of organic fertilizer (F0), TIB-440060 presented the lowest levels in antioxidants, polyphenols and carotenoids. The highest levels are recorded on plots receiving annually (F1) 20 t/ha of organic fertilizer. Those receiving the same quantities biennially (F2) of organic fertilizer presented highest levels of antioxidants and polyphenols. Differences were less obvious for total carotenoids between annual (F1) and biennial (F2) inputs contributions of organic fertilizer. Making comparisons of different doses of organic fertilizer in tables 4 and, 5 increases of 19.61%, 34.41% and 12.82% respectively in TAC, TPC and TCC due to annual contributions (F1) were observed. These increases were respectively 15.94%, 15.46% and 12.17% in TAC, TPC and TCC when these inputs of organic fertilizer are conducted biennially (F2).

These results suggested that the contribution of organic fertilizer had a positive effect on the production of total antioxidants, polyphenols and carotenoids. Frequent inputs of organic fertilizer produced the best results. However, biennial organic contributions could still cause a significant accumulation of total carotenoids in TIB-440060 tubers.

Effect of mineral fertilization: the effect of different doses of mineral fertilizers (table 1) indicated a significant variation ($P < 0.001$) (table 3) in accumulated TAC, TPC and TCC of TIB-4400060. Results obtained indicated that TAC were influenced by the combined effects of nitrogen and potassium, as showed for T2 and T3 (absence of phosphorus) with values of 3.107 and 3.378 mg/g of dw respectively (table 6). Lowest levels of antioxidants were observed in the presence or absence of nitrogen-phosphorus-potassium. But, for polyphenols, highest levels were obtained in formulations combining phosphorus at a minimal dose of 30 kg/ha, and the lowest levels were recorded when phosphorus was absent. The biosynthesis of polyphenols seemed to be positively influenced by phosphate fertilizers. Soils of Burkina Faso recognized for their poverty in phosphorus (Dembélé et al., 1991) could constitute a limit for the accumulation of polyphenols in OFSP. Supplies of phosphate fertilizer at a minimal dose of 30 kg/ha could be a solution for an improvement of polyphenolic accumulation in OFSP. In addition, total carotenoid contents did not clearly reflect the influence of different doses of mineral fertilizers on their production

Antioxidants, polyphenols and carotenoids biosynthesis would depend jointly on the composition of soil in nitrogen, phosphorus and potassium.

Effect of organo-mineral fertilization: TAC, TPC and TCC from the TIB-440060 OFSP tubers varied significantly ($P < 0.001$) (table 3) in relation with organo-mineral fertilization doses used. In general, combinations using minimal doses about 15 kg/ha of nitrogen, 30 kg/ha of phosphorus and 45 kg/ha of potassium (T2, T3, T5, T7, T8 and T9) associated with an annual (F1) or biennial (F2) contribution of 20 t/ha of organic fertilizer gave the highest values of total antioxidant, polyphenolic and carotenoid concentrations. These results indicated that the impact on the levels of antioxidants is noticeable when fertilizers used are sufficient to allow a synergistic action contributing to raise TAC values. The table 8 showed the contribution (percent) due to the individual effects of the organic fertilizer, mineral fertilizers and the combined effects of organic and mineral fertilizers on the accumulation of total antioxidants, polyphenols and carotenoids in TIB-440060 OFSP tubers. Then, the use of only organic fertilizer was responsible for 14.85%, 21.98% and 4.38% increases of TAC, TPC and TCC respectively. These increases were about 35.36%, 7.35% and 29.50% respectively when using individual mineral fertilizers. However, for an association of organic and mineral fertilizers increases were respectively about 44%, 68.39% and 62.63% for a maximal production of antioxidants, polyphenols and carotenoids in the studied OFSP tubers. These results highlight the importance of organo-mineral fertilization in the accumulation of phytochemicals contributing to a good nutritional quality. In addition, a strong positive correlation observed between the three parameters studied suggests a positive link between them and the efficiency of the Folin-Ciocalteu method used for their quantification.

CONCLUSION

This study, which is one of the first in West Africa, allowed to highlight the effects of organo-mineral fertilization on the nutritional quality the orange-fleshed sweetpotato. It is clear that the individual use of organic manure and mineral fertilizers have a positive effect on the production of antioxidants, polyphenols and total carotenoids. However, the major effects were obtained by an association of the two types of fertilizer. Annual inputs of organic fertilizer produce effects on the studied parameters. But when carotenoids-rich TIB-440060 OFSP tubers were targeted, biennial inputs of organic fertilizer could be recommended. The accumulation of antioxidants was positively influenced by the binary fertilizers containing nitrogen and potassium; the production of polyphenols was influenced by the phosphate fertilizer while the production of carotenoids was not affected from mineral fertilizers.

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Table 1: Details of 9 combinations mineral-based nitrogen (N), phosphorus (P) and potassium (K)

Traitement		**Nitrogen** (kg-N ha^{-1})	**Phosphorus** (kg-P_2O_5 ha^{-1})	**Potassium** (kg-K_2O ha^{-1})
T1	$N_0P_0K_0$	0	0	0
T2	$N_{15}P_0K_{45}$	15	0	45
T3	$N_{30}P_0K_{90}$	30	0	90
T4	$N_{60}P_0K_{100}$	60	0	100
T5	$N_0P_{30}K_{100}$	0	30	100
T6	$N_{30}P_{30}K_0$	30	30	0
T7	$N_{15}P_{30}K_{45}$	15	30	45
T8	$N_{30}P_{30}K_{90}$	30	30	90
T9	$N_{60}P_{30}K_{100}$	60	30	100

Table 2: The twenty-seven (27) organo-mineral fertilizer combinations tested on sweetpotato the variety TIB-440060

Organic Fertilization	Mineral Fertilization								
F_0=0	$N_0P_0K_0$ **T1**	$N_{15}P_0K_{45}$ **T2**	$N_{30}P_0K_{90}$ **T3**	$N_{60}P_0K_{100}$ **T4**	$N_0P_{30}K_{100}$ **T5**	$N_{30}P_{30}K_0$ **T6**	$N_{15}P_{30}K_{45}$ **T7**	$N_{30}P_{30}K_{90}$ **T8**	$N_{60}P_{30}K_{100}$ **T9**
F_1	T4	T8	T9	T1	T6	T2	T5	T3	T7
F_2	T8	T7	T1	T3	T6	T2	T4	T9	T5

F0: Control (without organic fertilizer); F1: application of 20 t / ha of organic fertilizer annually. F2: application of 20 t/ha every two years

Table 3: ANOVA of Means Square for phytochemical compound in TIB-440060 tubers

	TAC	TPC	TCC
Organic fertilization (O)	1,994***	2,447***	0,00004**
Mineral fertilization (M)	1,187***	0,205***	0,00006***
Organo-mineral fertilization (OxM)	0,745***	0,952***	0,00007***

** pour P=0.008, *** pour P<0.001

Table 4: Influence of different doses of organic fertilization on the production of TAC, TPC and TCC in TIB-440060 tubers

	TAC (mg/g)	**TPC (mg/g)**	**TCC (mg/g)**
F_0	2.606	1.749	0.016
F_1	3.118	2.351	0.018
F_2	3.022	2.059	0.018
standard Error	0.027	0.041	0.0004
LSD	0.074	0.115	0.001

Table 5: Increase of antioxidant, polyphenolic and total carotenoid contents on the OFSP variety TIB-440060 according to periodicity of organic manure application

Organic fertilizer	F1	F2
Rate of increase (TAC)/F0	19.61 %	15.94 %
Rate of increase(TPC)/F0	34.41 %	17.69 %

Rate of increase(TCC)/F0	12.82%	12.17 %

F0: Control (without organic fertilizer); F1: application of 20 t / ha of organic fertilizer annually. F2: application of 20 t/ha every two years

Table 6: Influence of different doses of mineral fertilization on the production of TAC, TPC and TCC in TIB-440060 tubers

	TAC(mg/g)	**TPC(mg/g)**	**TCC(mg/g)**
T_1	2.736	1.983	0.019
T_2	3.107	2.126	0.016
T_3	3.378	1.877	0.013
T_4	2.996	1.916	0.019
T_5	2.953	2.191	0.013
T_6	2.213	2.128	0.019
T_7	2.940	2.184	0.017
T_8	2.324	1.838	0.020
T_9	2.950	2.237	0.016
standard Error	0.027	0.042	0.0004
LSD	0.073	0.115	0.001

Table 7: Levels of antioxidants, total polyphenols and carotenoids accumulated by the OFSP variety TIB-440060 under different organo-mineral combination

Fertilization	Phytochemicals		
	TAC (mg/g)	TPC (mg/g)	TCC (mg/g)
F_0T_1	2.076 ± 0.049^{bc}	1.234 ± 0.018^{a}	0.011 ± 0.001^{abc}
F_0T_2	2.622 ± 0.006^{def}	1.662 ± 0.023^{bcde}	0.014 ± 0.001^{cdef}
F_0T_3	3.199 ± 0.080^{ghi}	1.720 ± 0.016^{cde}	0.016 ± 0.000^{fgh}
F_0T_4	3.209 ± 0.061^{ghi}	2.442 ± 0.106^{jkl}	$0.016\pm2.E05^{efg}$
F_0T_5	1.641 ± 0.088^{ab}	1.415 ± 0.035^{abc}	$0.011\pm2.E05^{abc}$
F_0T_6	2.303 ± 0.039^{cd}	2.632 ± 0.052^{lm}	0.015 ± 0.000^{def}
F_0T_7	2.565 ± 0.100^{de}	1.864 ± 0.084^{ef}	0.018 ± 0.000^{ghij}
F_0T_8	3.101 ± 0.042^{fghi}	1.373 ± 0.142^{ab}	0.018 ± 0.000^{ghij}
F_0T_9	2.736 ± 0.050^{defg}	1.396 ± 0.034^{ab}	0.018 ± 0.001^{fghi}
F_1T_1	3.215 ± 0.044^{ghi}	2.567 ± 0.148^{lm}	0.022 ± 0.001^{kl}
F_1T_2	3.336 ± 0.160^{hij}	2.510 ± 0.101^{klm}	0.023 ± 0.000^{l}
F_1T_3	3.219 ± 0.147^{ghi}	1.863 ± 0.031^{ef}	0.008 ± 0.001^{a}
F_1T_4	3.216 ± 0.057^{ghi}	1.913 ± 0.049^{efg}	0.019 ± 0.000^{hijk}
F_1T_5	2.557 ± 0.269^{cde}	2.351 ± 0.016^{ijkl}	0.019 ± 0.00^{ghij}
F_1T_6	2.875 ± 0.171^{efgh}	2.126 ± 0.150^{fghi}	0.021 ± 0.000^{jkl}
F_1T_7	3.496 ± 0.183^{ij}	2.962 ± 0.038^{n}	0.013 ± 0.000^{cde}
F_1T_8	3.455 ± 0.097^{ij}	2.632 ± 0.115^{lm}	0.018 ± 0.000^{ghij}
F_1T_9	2.684 ± 0.157^{def}	2.233 ± 0.060^{hijk}	0.011 ± 0.000^{abc}
F_2T_1	2.916 ± 0.122^{efgh}	2.147 ± 0.052^{fghij}	0.023 ± 0.000^{l}
F_2T_2	3.362 ± 0.112^{hij}	2.204 ± 0.035^{ghij}	0.009 ± 0.001^{ab}
F_2T_3	3.714 ± 0.023^{j}	2.045 ± 0.056^{fgh}	0.012 ± 0.000^{bcd}
F_2T_4	2.562 ± 0.017^{cde}	1.391 ± 0.013^{ab}	0.018 ± 0.000^{ghij}
F_2T_5	3.580 ± 0.077^{ij}	2.804 ± 0.066^{mn}	0.008 ± 0.000^{a}
F_2T_6	1.459 ± 0.025^{a}	1.625 ± 0.044^{bcde}	$0.019\pm8.E05^{ijk}$
F_2T_7	2.757 ± 0.047^{defg}	1.724 ± 0.009^{de}	0.019 ± 0.001^{hijk}
F_2T_8	3.414 ± 0.151^{ij}	1.506 ± 0.020^{abcd}	0.023 ± 0.000^{l}
F_2T_9	3.429 ± 0.254^{ij}	3.077 ± 0.011^{n}	0.021 ± 0.001^{jkl}

Data are expressed as means ± SE of triplicate experiments. Means in a column not having a common letter are different (P<0.05).

Table 8: contribution (per cent) of the type of fertilization on the accumulation of TAC, TPC and TCC in TIB-440060 tubers

Type of fertilization	Contribution (%)		
	TAC	TPC	TCC
Organic fertilization	14.85	21.98	4.38
Mineral fertilization	35.36	7.35	29.50
Organo-mineral fertilization	44.38	68.39	62.63
Residual	5.41	2.28	3.49

Table 9: Correlation (R^2) between the different quality parameters in sweetpotato

	TPC	TAC
TAC	0.8947	
TCC	0.7885	0.9784