Full Length Research Paper

Comparative evaluation of organic and inorganic fertilizers on total phenolic, total flavonoid, antioxidant activity and cyanogenic glycosides in cassava (*Manihot esculenta*)

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The study evaluated the effects of fertilizer sources and varieties on total phenolic, total flavonoid, antioxidant activities and cyanogenic glycosides of cassava leaves and tuber. The main factor was fertilizer sources (vermicompost, empty fruit bunch compost, NPK 15:15:15) and sub-factor was based on cassava varieties (Medan and Sri Pontian). Treatments were arranged in a split plot design with three replications. Phytochemical compounds and the antioxidant activities in young leaves and tuber were carried out after harvesting. Total phenolic and flavonoid compounds were determined by using Folin-Ciocalteu assay and aluminium chloride colorimetric method, respectively. The fertilizer sources and varieties were found to have significant effect on phytochemical compounds. Fertilizer and variety interaction was significant in total phenolics, total flavonoid, 1,1-diphenyl-2-picryl-hydrazyl (DDPH) scavenging assay and ferric reducing antioxidant capacity (FRAP) scavenging assay for tuber and leaves. The phenolic and flavonoid content was significantly higher (P<0.01) in the vermicompost treatments compared to mineral fertilizer and EFB compost. Medan had higher antioxidant activities as well as total content of phenolics and flavonoid in comparison with Sri Pontian. Organic fertilizer application resulted in significantly lower cyanide content compared to inorganic fertilizer. The results indicate that the fertilizer sources change phytochemical compounds of cassava tuber and leaves.

Key words: *Manihot esculenta*, cassava tuber, cassava leaves, fertilizer sources, phytochemical compounds, antioxidant activity.

INTRODUCTION

Cassava (*Manihot esculenta*), also called mandioca, manioc, yucca or tapioca is a perennial shrub of the family Euphorbiaceae, cultivated mainly for its starchy roots (Julie et al., 2008). It is the world's sixth most important crop in terms of production and is a vital staple food to over 500 million in the humid tropics (Reilly et al.,

2007). The tuber is popularly used for production of tapioca chip and starch. Starch is used mainly in the manufacture of monosodium glutamate, glucose and paper products. Tubers are also processed as feed for livestock. New food uses of cassava include flour in gluten free or gluten-reduced products (Falade and Akingbala, 2011).

Cassava leaves are rich in iron, zinc, manganese, magnesium, calcium, vitamins B1, B2 and C, and carotenoids (Adewusi and Bradbury, 1993; Wobeto et al., 2006). Furthermore, the essential amino acid profile of the leaves is higher than the Food and Agriculture Organization's recommended reference protein intake

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Abbreviations: DDPH, 1,1-Diphenyl-2-picryl-hydrazyl; **FRAP,** ferric reducing antioxidant capacity.

and to that of soybean protein (FAO/WHO, 1973; Julie et al., 2008). Cassava leaves also contain moderate level of phytochemicals that are important as natural antioxidant components of plant food products. Even though cassava is an important food, it contains toxic and antinutritional substances that interfere with digestion and uptake of nutrients (Wobeto et al., 2007). Cassava contains potentially toxic levels of cyanogenic glycosides, made up of linamarin (95% of total cyanogens content) and lotaustralin (5%). Linamarin is present in all cassava tissues. Cyanogenic glycosides may be defined chemically as glycosides of the α-hydroxynitriles and are secondary metabolites produced by plants. Natural antioxidants or phytochemical compounds are secondary metabolites of plants (Walton and Brown, 1999). The levels of phytochemical in plants depend on several factors including cultivar, storage condition, maturity stage and agricultural practices (organic or conventional) (Perez-Lopez et al., 2007).

Fertilization has been reported to have influence on phytochemical status of crops. Inorganic fertilizer is found to reduce the antioxidant level while organic fertilizer was proven to enhance the antioxidant content in plant (Dumas et al., 2003). Genotypic differences are the main factor that causes a large variation in vitamin content, antioxidant capacity and phenolic content (AVRDC, 2003; Rekika et al., 2005). According to Bok et al. (2006), different varieties will give a different level of antioxidant compound. In white clover, immaturity, moisture stress, low light intensity, cool temperature, and inadequate P supply were shown to favour high cyanide content (Vickery et al., 1987).

There are studies on various crops that have compared the levels of antioxidant compounds in organically and conventionally grown products. Asami et al. (2003) reported that there were significantly higher total phenolics in organically grown marrionberries (620 mg/100 g fresh weight) as compared to conventional method (412 mg/100 g). According to Benbrook (2005), organic farming elevated antioxidant levels in about 85% of the cases studied, on average level is higher by 30% compared to the food grown conventionally whereas Perez-Lopez et al. (2007) reported that organic farming had a significant effect on nutritional content in peppers, increasing the vitamin C activity, total phenolic compounds and carotenoid contents.

Organically grown vegetables are gaining popularity among health conscious consumers and food retailers. The uses of organic fertilizers which are easily available locally are not only economic but also ensure continuous productivity without causing any potential long term threat to environment and health. Since health conscious consumers are interested in optimizing the nutritional composition with minimal chemical residual of foods, this study was carried out with the objective to determine the effect of inorganic and various organic fertilizer sources on the antioxidant activity in the leaves and tuber of two cassava varieties namely Medan and Sri Pontian. These two varieties had been selected as there are commercially grown for cassava chips as well as have low cyanide content.

MATERIALS AND METHODS

The factorial experiment was conducted on sandy clay loam soil pH 5.7 under open field condition from January 2010 to October 2010. The treatments were arranged in a split plot design with three replications. The main plot was fertilizer sources consisting of vegetable waste vermicompost (N: 2.32%; P: 1.54%; K: 1.06%), empty fruit bunch compost (N: 1.46%; P: 1.47%; K: 2.58%) and inorganic fertilizer (N: 15%; P: 15%; K: 15%). The sub plot was cassava Medan and Sri Pontian varieties. 20 to 25 cm length stem cuttings were planted at 1 × 1 m. There were 2 m gaps between treatments and 1 m alleys between plots. The amount of fertilizer applied was calculated based on 180 kgh^{-1} of K_2O. The soil was thoroughly plowed and mixed with organic compost during planting while inorganic fertilizer was applied equally at two and twenty weeks after planting. The soil was covered with organic mulch and sprinkler irrigated. The tubers and fresh young leaves were harvested after nine months and analyzed for total phenolic acids, total flavonoids, cyanogenic glycosides contents, and DPPH free radical and FRAP scavenging assays. The data were analyzed using analysis of variance and significant differences between means were done by least significant difference test ($p<0.05$)

Extraction of total phenolic acids and total flavonoids

Extraction of total phenolic acid and total flavonoid assay were conducted using modified method of Marinova et al. (2005). Cassava roots and leaves weighing 0.5 g, respectively were ground using mortar and pestles. All samples were homogenized with 50 ml distilled water and transferred into covered flask. Then, the mixtures were centrifuged for 5 min at 14000 rpm. The supernatant was collected and used for total phenolic acids and total flavonoids quantification.

Total phenolics

Total phenolic (TP) compounds were conducted as described by Marinova et al. (2005) using the Folin-Ciocalteu assay. 1 ml extracts was added into a flask containing 9ml of distilled water. Then 1 ml of Folin-Ciocalteu's phenol reagent was added and the mixture was mixed thoroughly. After 5 min, 10 ml of 7% sodium carbonate was added. The mixture was diluted to 25 ml with the addition of 4 ml of distilled water and allowed to stand at room temperature for 90 min. The absorbance was monitored using a spectrophometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) at 750 nm. TP content was expressed as mg gallic acid equivalents (GAE)/g samples.

Total flavonoid assay

Total flavonoid was conducted according to Marinova et al. (2005) using aluminium chloride colorimetric method. 1 ml of extracts was added with 4 ml of distilled water in a flask. Then, 0.3 ml of 5% $NaNO_2$ was added. After 5 min, 0.3 ml of 10% $AlCl_3$ was added and after 6 min, 2 ml of 1 M NaOH was added. The mixture was diluted to 10 ml with distilled water. The absorbance of the solution was measured at 510 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). The results were expressed as mg catechin equivalents (CE)/g samples.

Table 1. ANOVA of means square [MS(Pr>F)] for phytochemical compound in tuber.

Source	Total phenolic	Total flavonoid	DPPH scavenging assay	FRAP assay	Cyanogenic glycoside
Fertilizer	259.23*	53.76**	502.98**	672.65**	0.022*
Variety	134.48*	5.44*	494.02**	0.37^{ns}	0.002^{ns}
F x V	56.91*	17.43**	45.34**	13.31*	0.001^{ns}
CV(%)	2.91	2.75	3.68	3.55	9.02

ns **, *, non significant or significant at P≤0.01, P≤0.05, respectively.

Table 2. ANOVA of Means Square [MS(Pr>F)] for phytochemical compound in leaves.

Source	Total phenolics	Total flavonoids	DPPH scavenging assay	FRAP assay	Cyanogenic glycoside
Fertilizer	51.52**	3.00**	2373.32**	1593.14**	0.02**
Variety	9.49**	0.308**	144.06**	33.04**	0.0001^{ns}
F x V	2.79**	0.98**	82.85**	8.81**	0.0008^{ns}
CV(%)	4.45	3.11	0.82	0.74	5.29

ns, **, non significant or significant at P≤0.01, respectively.

Extraction of antioxidant compounds

Extraction of antioxidant compounds was conducted employing the method modified by Wong et al. (2006). Fresh samples (0.5 g) of tuber and leaf were cut into small pieces at placed in 150 ml conical flask. A total volume of 25 ml of distilled water was added and covered with aluminum foil. The conical flasks containing the samples were placed in orbital shaker for 1 h in the dark at room temperature. Then, the samples were filtered using Whatman No. 1 paper. The extracts were stored at 0 to 4° C before analysis.

DPPH free radical scavenging assay

1,1-diphenyl-2-picryl-hydrazyl (DDPH) was purchased from Sigma-Aldrich (USA). The antioxidant activities DPPH was performed according to Wong et al. (2006) procedures with some modifications. The initial absorbance of DPPH in methanol was measured at 515 nm until the absorbance remains constant. 40 µl of extracts was added to 3 ml alcohol solution of DPPH (0.1 mM). The samples were first kept in a dark place at room temperature and after 30 min the absorbance was measured using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) at 515 nm. The percent of inhibition was determined using the formula: percent of inhibition (%) = [(A515 of control-A515 of sample)/A515 of control] x 100.

Ferric reducing antioxidant power assay (FRAP)

The determination of the total antioxidant activity using FRAP assay in the extract followed after a modified method reported by Wong et al. (2006). 200 µl of extract was added to 3 ml of FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM FeCl.6H$_2$0 solution) and the reaction mixture was incubated in a water bath at 37°C for 30 min. The increase in absorbance was measured at 593 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). The percent of antioxidant was calculated using the formula: percent of antioxidant (%) = [(A593 of sample-A593 of control)/A593 of sample] x 100.

Determination of cyanogenic glycoside

The alkaline pictrate method of Sarkiyayi and Agar (2010) was used to determine cyanogenic glycoside in cassava. 5.0 g of roots and leaves were weighed and dissolved in 50 ml distilled water in conical flasks. The mixtures were allowed to stay overnight and filtered. The filtrates were collected, and 4 ml of alkaline pictrate solution was added to each and incubated in water bath for 15 min. When the mixture turned to reddish brown, the absorbance was taken at 490 nm using a spectrophometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Different concentration of hydrogen cyanic acid (HCN) was prepared containing 0.02 to 0.10 mg/ml cyanide for blank mixture. The measurement of cyanogenic glycoside was repeated in triplicates. The cyanide content was extrapolated from cyanide curve.

RESULTS AND DISCUSSION

The phytochemical compounds in methanol extracts of tuber and leaves are presented in Tables 1 and 2, respectively. Fertilizer type and cassava varieties showed significant effect on the antioxidant compounds measured.

The total phenolic content was measured by Folin Ciocalteu reagents in terms of gallic acid equivalent (standard curve equation: y= 1.961 - 0.001x, R^2= 0.553). There was significant difference (P≤0.01) in total flavonoid content of tuber and leaves treated with various fertilizer sources (Tables 3 and 4). In both plant parts, the highest total phenolic content was found from plants treated with vermicompost, followed by empty fruit bunch compost and inorganic fertilizer. The phenolic content was found to increase as much as 30% in tuber and 26% in leaves in response to vermicompost compared to inorganics. Similar response was also obtained in total flavonoid content. In this respect, tubers and leaves of

Table 3. Phytochemical compounds in tuber of cassava varieties as affected by fertilizer sources.

Source	Total phenolics (mg GAE/g fw)	Total flavonoids (mg CE/g fw)	DPPH scavenging assay (%)	FRAP assay (%)	Cyanogenic glycoside (mg/100 g fw)
Fertilizer source					
VWV	10.88[a]	2.71[a]	50.81[a]	46.10[a]	0.40[b]
EFBC	9.44[b]	2.32[b]	43.60[b]	40.38[b]	0.42[b]
Inorganic	8.35[c]	2.18[c]	32.64[c]	25.58[c]	0.51[a]
Variety					
Medan	9.83[a]	2.47[a]	47.58[a]	37.50[a]	0.43[a]
Sri Pontian	9.20[b]	2.30[b]	37.11[b]	37.21[a]	0.45[a]

Means with same letter are not significantly different by LSD, at 5%.

Table 4. Phytochemical compounds in leaves of cassava varieties as affected by fertilizer sources.

Source	Total phenolics (mg GAE/g fw)	Total flavonoids (mg CE/g fw)	DPPH scavenging assay (%)	FRAP assay (%)	Cyanogenic glycosides (mg/100 g fw)
Fertilizer source					
VWV	11.51[a]	2.47[a]	67.30[a]	68.11[a]	0.49[b]
EFB	10.02[b]	2.07[b]	54.70[b]	54.45[b]	0.52[b]
Inorganic	9.14[c]	1.90[c]	44.37[c]	50.08[c]	0.61[a]
Variety					
Medan	10.53[a]	2.26[a]	57.65[a]	59.06[a]	0.54[a]
Pontian	10.67[a]	2.22[ab]	53.22[b]	55.50[b]	0.55[a]

[z]Means with same letter are not significantly different by LSD at 5%.

Table 5. Correlation coefficients between total phenolic compounds (TPC), total flavonoid content (TFC) and antioxidant activity determination assays (DPPH and FRAP) of cassava.

Parameter	TPC	TFC	DPPH	FRAP
TPC	-	0.62*	0.83**	0.82**
TFC	-	-	0.61[ns]	0.74**
DPPH	-	-	-	0.79**
FRAP	-	-	-	-

For correlation coefficients, n=18; ns, *, **, non significant or significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

vermicompost plant had 24 and 30% higher flavonoids respectively than inorganics. Differences of compost and vermicompost on their influences on physiological activities could be due to the fundamental differences between the composting and vermicomposting processes enzymatic activity in vermicompost and the presence of beneficial worm in VWV (Atiyeh et al., 2000). According to Benbrook (2005), organic farming elevated antioxidant levels in about 85% of the cases study, and on average, levels were about 30% higher compared to food grown conventionally.

The significant positive correlation shown in Table 5 (r = 0.62) between total phenolic and flavonoid compounds indicates that an increase in phenolic was followed by an increase in total flavonoid. Both were found to be highly correlated with antioxidant activity. Among Medan and Sri Pontian, it was found that Medan had higher total phenolics and total flavonoids. Phenolic compounds and flavonoids in this condition may play an important role as scavengers for free radicals and other oxidative species (Giorgi et al., 2009). Phenolic molecule is characteristic of a plant species or even of a particular organ or tissue of the plant (Karakaya, 2004). They are closely related to the secondary and nutritional quality of foods derived from plant resource. Phenolic compounds, at low concentration may act as an antioxidant and protect food from oxidative deterioration (Karakaya et al., 2001). At high concentration, they may interact with proteins, carbohydrates and minerals (Karakaya, 2004).

Several methods have been developed to monitor the total antioxidant capacity in biological samples. These assays differ in how the different radicals and target molecules are generated and in the way the end points are measured. Because of this, more than one method is
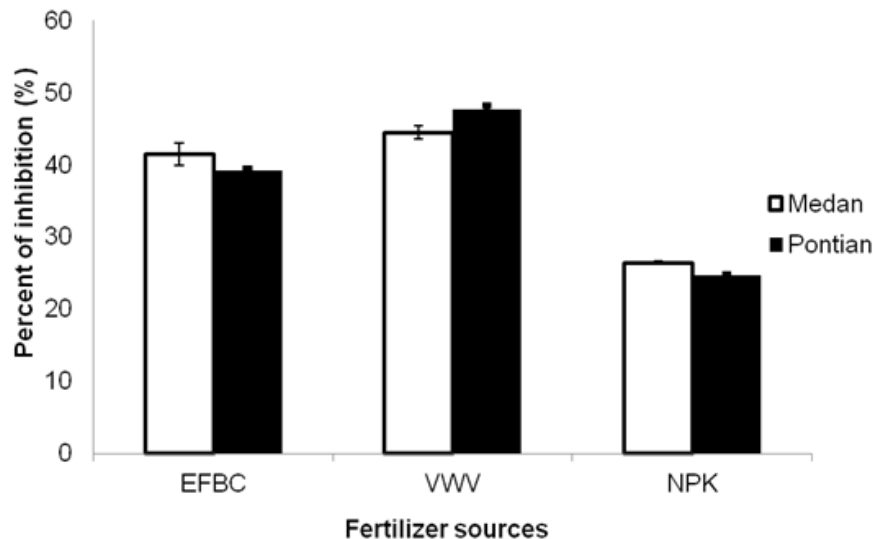
Figure 1. Total antioxidant content of cassava tuber treated with different fertilizer sources expressed as percent of inhibition using DPPH method.

required to investigate the *in vitro* antioxidant potential of complex mixtures such as fruit extracts (Lamien-Meda et al., 2008). Therefore, 1,1-diphenyl-2-picryl-hydrazyl (DDPH) and ferric reducing antioxidant capacity (FRAP) assays were used to evaluate antioxidant activity in this study. DPPH is suggested by many authors because this method is repeatable and provides a precise assay for measuring the antioxidant activity (Liu et al., 2008). Generally tuber and leaves of Medan cassava had higher DPPH scavenging activity whereas in response to fertilizer sources, vermicompost treatment resulted in the highest DPPH scavenging activity (Table 3 and 4). However, results also indicate that there was significant difference (P≤0.01) interaction effects observed in DPPH scavenging activity.

The percent inhibition of the DPPH radical as a function of the antioxidant concentrations is shown in Figures 1 and 2. In the tuber, Pontian variety showed the highest scavenging activity (50.81%) only in response to application of vermicompost but with other fertilizer sources, Medan variety exhibited superior responses (Figure 1). Whereas in the leaves, Medan variety treated with organic fertilizers recorded highest percentage of inhibition but was not significantly different under inorganic treatment (Figure 2). In this study, the results show that percentage inhibition of the leaf extracts were higher than in tuber. Higher percentage of inhibition from leaves extracts has been associated with higher total phenolics. This indicate that phenolic compounds were the main contributors of antioxidant activity (Bala Nambisan et al., 2012). Based on the results obtained, it is possible that several compounds of different polarities may contribute to the antioxidative properties of cassava tuber and leaves. Kirakosyan et al. (2003) reported

that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals.

The ferric reducing ability (FRAP assay) is widely used in the evaluation of the antioxidant in dietary polyphenol (Luximon-Ramma et al., 2005). In the present study, it was observed that FRAP assay of the methanolic extracts of the tuber had higher activity differences in Pontian and Medan with application of vermicompost and NPK fertilizer (Figure 3). As can be seen, the effect of NPK fertilizer on FRAP assay for leaves part was lower than in both compost (Figure 4). Difference in FRAP values of Medan and Pontian of leaves part was found only with NPK application. In this study, results suggest that percent of antioxidant in tuber part were less than leaves. This is probably due to nutrients distribution in the plant. Roots have very low quantities of lipids, minerals, proteins, and vitamins compared to the leaves (Julie et al., 2008). The effect of antioxidants on FRAP was estimated from their ability to reduce 2,4,6-tripyridyl-*s*-triazine (TPTZ)-Fe (III) complex to TPTZ-Fe (II). Antioxidant activity is found to be highly correlated with phenolic contents (Table 5). According to (Oktay et al. 2003), it was a trend in many plant species, which shows a strong positive relationship between total phenolic contents and antioxidant activity. The mechanism of action of DPPH and FRAP are different, i.e. scavenging of DPPH cation radicals in the DPPH assay and reduction of ferric ions in the FRAP assay.

The maximum recommended cyanide levels in foods established by Codex Alimentarius Commission of the FAO/WHO is10 mg CN equivalents/kg dry weight. Linamarin is present in large amounts in the leaves and the peel of the roots (900 to 2000 mg HCN equivalents
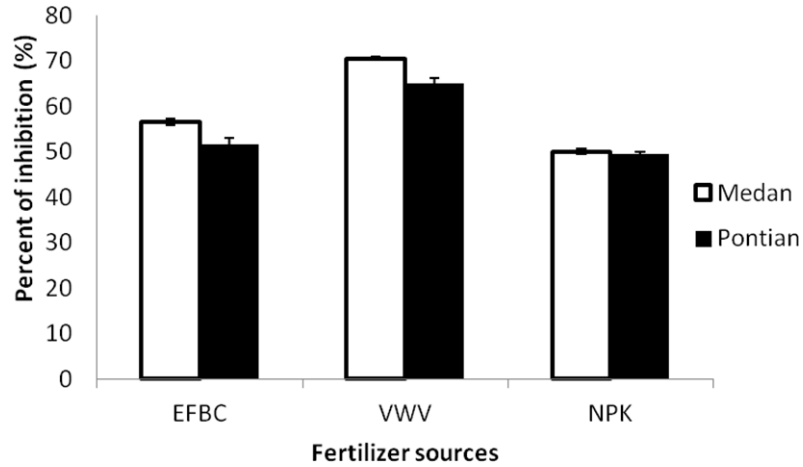
Figure 2. Total antioxidant content of cassava leaves treated with different fertilizer sources expressed as percent of inhibition using DPPH method.
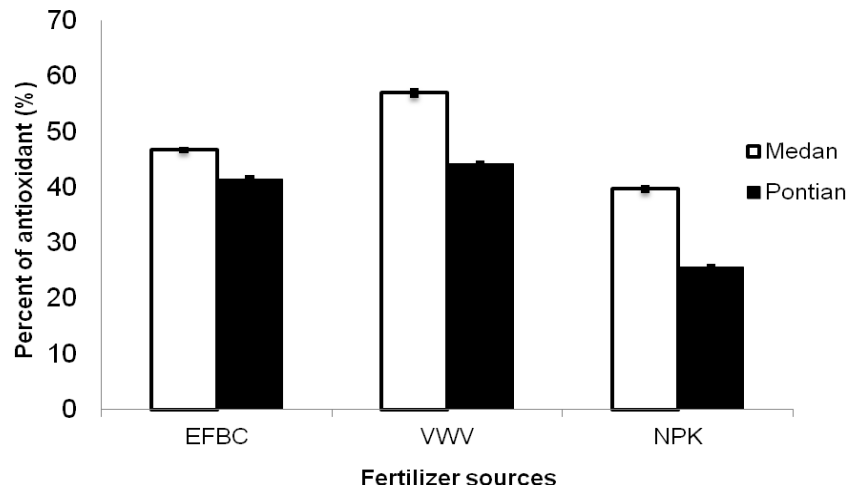

Figure 3. Total antioxidant content of cassava tuber treated with different fertilizer sources expressed as percent of antioxidant using FRAP method.
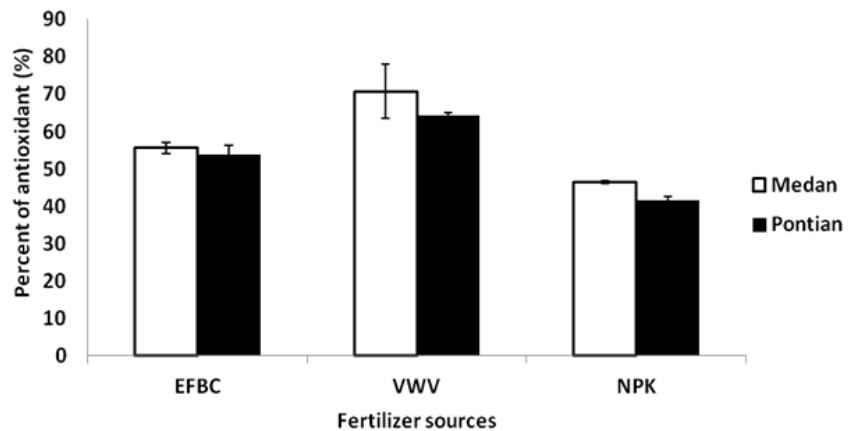

Figure 4. Total antioxidant content of cassava leaves treated with different fertilizer sources expressed as percent of antioxidant using FRAP method.

kg^{-1} fresh weight (Nhassico et al., 2008). In this experiment, the amount quantified in both plant parts was considerably low and there was no significant interaction effect of fertilizer sources x cassava variety on level of cyanogenic glycosides for both tuber and leaves (Tables 1 and 2). Application of inorganic fertilizer was observed to increase the level of cyanide content in tuber as well as leaves part. In general, organic fertilizer application resulted in significantly lower cyanide content compared to inorganic fertilizer for tuber part. Similar results have been obtained for leaves part applied with organic fertilizer (Table 4). The low content of these anti-nutrients using organic fertilizer would therefore permit the absorption of these elements which they form complexes with.

Conclusion

The nutritive value of cassava is largely dependent on fertilizer treatments and genotype. Fertilization is the most important and controllable factor affecting nutritional value of fruit and vegetables. The results indicate that application of vermicompost and empty fruit bunch compost can enhance antioxidant activities of field grown cassava. Generally, the level of the total phenolic acid content in tuber and leaves was increased by application of organic fertilizer. The application of inorganic fertilizer had increased the level of cyanogenic glycoside in both plant parts. Vermicompost is favourable for the enhancement of antioxidants in cassava, so it can contribute to the improvement of nutritional value of tuber and its products and leaves as vegetable. However, when application of inorganic fertilizer was used, phytochemical contents tend to decrease. Therefore, replacing inorganic with organic fertilizer can improve nutritional value of fertilized cassava as food product as well as for the environment. Organic fertilizer should be used in place of chemical fertilizer for environmentally sustainable production of better quality cassava.

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