Hydrolyzed Organic Fish Fertilizer and Poultry Litter Influence Total Phenolics and Antioxidants Content but Not Yield of Amaranth, Celosia, Gboma, and Long Bean

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Abstract. Dietary intake of a variety of vegetables is very important for disease prevention and may help in the treatment of certain maladies. Experiments were conducted to evaluate yield and the content of antioxidants and phenolics of vegetable Amaranth (*Amaranthus hybridus*), Celosia (*Celosia argentea*), Gboma (*Solanum macrocarpon*), and Long Bean (*Vigna unguiculata*) in response to poultry litter (PL) and a hydrolyzed fish fertilizer [Megabloom (MB)]. The experiments were conducted as a randomized complete block design with a four × three factorial treatment and four replications. The treatments were applied based on soil test recommendations in a single band 15 to 20 cm away from the plants 1 week after transplanting. Plants were harvested sequentially during the season as foliage and fruits became physiologically mature and once over at the end of the season. Species were analyzed for vitamin C content, total phenolics, and β-carotene content as well as antioxidant capacity. Organic amendments did not significantly influence biomass production, whereas species impacted fresh and dry biomass. Amaranth, Celosia, and Long Bean produced greater fresh and dry biomass than did Gboma. Vitamin C content was highest among Amaranth and Celosia plants receiving nitrogen–phosphorus–potassium (NPK) compared with the other two treatments, whereas that of Gboma was higher among plants receiving MB.

MB and PL enhanced β-carotene in Amaranth compared with NPK but that of Celosia and Gboma was enhanced by MB and NPK fertilizer. Total phenolic content was higher among Amaranth plants receiving NPK, whereas those for Long Bean were greater among plants receiving MB or NPK fertilizer. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) activity was enhanced by MB, MB and NPK, or MB only among Amaranth, Celosia, Gboma, and Long Bean plants, respectively. These results suggest that the impact of organic

amendments on biomass production was marginal and that species exerted a greater influence. However, there appears to be an enhancement of total phenolics and DPPH activity in response to organic amendments.

Vegetable Amaranth (*Amaranthus hybridus*) (Mosha et al., 1995), Celosia (*Celosia argentea*) (Orhue, 2010), Gboma (*Solanum macrocarpon*) (AVRDC, 2002), and Long Bean (*Vigna unguiculata*) (Chitindingu, 2005; Mosha et al., 1995; Palada et al., 2006), are highly nutritious niche market specialty vegetables being evaluated for growth, adaptability, and production under Alabama conditions, including responses to different organic fertilizers. Amaranth and Celosia are leafy vegetables that are good sources of antioxidants and phenolics, are similar to spinach, and are commonly grown in parts of Africa and Asia (Chitindingu, 2005). Gboma is widely grown in Africa and Southeast Asia for its fruits as well as its leaves, which are similar to those of kale and are good sources of protein, fiber, vitamins,

and minerals (Schipper, 2000). Long Bean is popular in Asian countries, and its pods are similar to that of snap beans but are pencil thin and are harvested when immature before seed fill. Long beans have adequate levels of vitamin C, provitamin A, folate, and protein (Mosha et al., 1995).

Animal manures such as PL and hydrolyzed fish fertilizers are relatively less expensive than conventional inorganic fertilizers and readily available (especially PL). Manures are relatively good sources of plant nutrients and organic matter (Kpombrekou et al., 2002; Toor et al., 2006) and not only improve soil physical and chemical properties, but also enhance plant growth (Boyhan et al., 2010; Russo, 2010). For example, an incubation study evaluating nitrogen (N) availability from liquid fish protein-based organic fertilizers, Hartz (2010) showed that 79% to 83% of total N was available after 1 week and 83% to 99% after 4 weeks. In addition, PL and hydrolyzed fish fertilizers when used as organic amendments also increase disease and insect resistance, extended the shelf life of produce, and enhanced microbial activity (Davis and Riordan, 2004).

Russo (2010) evaluated the effects of the frequency of applied PL compared with an annual application of NPK on the production of bell peppers (*Capsicum annuum*), sweet corn (*Zea mays*), and cucumbers (*Cucumis sativus*). He found that an annual treatment with PL produced higher marketable yields of bell pepper and sweet corn compared with application in alternate years, whereas the opposite was true for cucumber.

Boyhan et al. (2010) reported that PL applications increased total yield of short-day onions but reduced that of medium bulbs. Adeoluwa and Cofie (2011) reported that vegetable Amaranth plants had a positive response to organic compost but the response varied with time and environmental conditions, whereas Jaipaul et al. (2010) reported that organic manures increased plant height of pepper and garden pea. Masarirambi et al. (2012) evaluated the effects of poultry manure applied at 20, 40, and 60 t·ha⁻¹ on lettuce production in South Africa and reported greater total and marketable yields at the 60-t·ha⁻¹ rate.

Plant responses to hydrolyzed fish fertilizers appear to be species-dependent. Schupp et al. (1993) reported that fish hydrolysate fertilizer reduced fruit set of ‘Delicious’ and ‘Golden Delicious’ apples. In contrast, Smagula and Dunham (1995) showed that fish hydrolysate fertilizer was as effective as 5N–10P–5K in raising leaf N, P, and K concentrations in prune and crop-year leaf samples as well as stem length and yield of low bush blueberry; fish hydrolysate is an acceptable alternative to soluble fertilizer for cranberries (DeMoranville, 1990).

Fruits and vegetables are critical parts of a healthy diet and are rich sources of many nutrients such as vitamins C and K, folate, thiamine, carotenes, several minerals, and dietary fiber (Palada et al., 2006). In addition, antioxidants that occur naturally in these fruits,

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vegetables, and whole grains are powerful weapons in combating inflammation and lowering heart disease and cancer risk (Odukoya et al., 2007).

Studies on organically grown produce have shown differences in micronutrient and phytochemical content, sometimes as high as 30% in contrast to crops conventionally grown (Riordan and Davis, 2005). Weibel (2000) harvested apples from five pairs of organic/integrated fruit farms with similar microclimate, soil conditions, and planting system and reported that contents of phenols (mainly flavonols) were 19% higher in organically grown apples. In contrast, Toor et al. (2006) reported that lycopene levels in tomato were reduced by the application of organic amendments. Zhao et al. (2007) reported that the levels of phenolic compounds in lettuce were consistently impacted by the nutrient source and suggested that this could have been related to general factors including the environment, season, and cultivar differences. Additionally, peppers and garden peas fertilized with organic manure have been previously shown to have higher vitamin C, total phenolics, and anthocyanin content (Abu-Zahra, 2011; Jaipaul et al., 2010). Similarly, broccoli treated with organic and bio-organic fertilizers produced antioxidants with greater chelating power (El-Moniem et al., 2012).

Although improved yield responses and higher phenolic concentrations have been reported in organically grown crops, these reports are generally inconsistent, because other factors may be involved (Carbonaro et al., 2002; Lombardi-Boccia et al., 2004; Sousa et al., 2005). For example, Young et al. (2005) suggested that organic systems may expose leafy vegetables to attack by insects resulting in higher concentrations of phenolics as plants produce them as a part of their defense mechanism.

Our objective was to evaluate the influence of a hydrolyzed organic fish fertilizer and PL on biomass production, total phenolic, and antioxidant content and DPPH activity of Amaranth, Celosia, Gboma, and Long Bean.

Materials and Methods

Experiments were conducted in a randomized complete block design with four $\times$ three factorial treatment arrangement and four replications. Each replication contained three plots with 10 plants each, the middle row of which was harvested. Seeds of all four species were sown in moist commercial Jiffy Mix (Jiffy Produces of America Inc., Batavia, IL) medium in TLC Pro-Trays transplant flats (TLC Polyform, Inc., Plymouth, MN) and covered with ≈ 0.6 cm of the medium. Flats were placed in a greenhouse watered as needed and seeds germinated within 5 to 7 d. Seedlings were grown for ≈ 6 weeks after which they were transplanted into three-row plots of 1.2 m $\times$ 6 m at the recommended plant spacing (Harrison et al., 2004; Mortley et al., 1992) for each species and moisture supplemented with drip irrigation.

Fertilizer treatments consisted of PL (20% N), hydrolyzed fish fertilizer (MB; 2% N), and conventional NPK. Fertilizer treatments for each species were based on soil test recommendations and were applied in a single band ≈ 15 to 20 cm away from the plants. Because no laboratory analysis was done on the PL, we used the average values of litter nutrients in similar waste management systems of Fulhage and Pfost (1994) based on incorporation 7 d or greater after collection.

Six inner plants from the middle row of each three-row plot were harvested periodically throughout the growing season (as green leafy foliage or pods). Succulent stems with intact leaves of ≈ 15 cm length (Amaranth and Celosia) or leaves (Gboma) were harvested every 2 weeks and the entire plant at the end of the growing season and fresh weights taken. Plant tissues were dried at 70 °C for 72 h and dry weights of component plant parts determined. Samples for antioxidant and phenolics analysis were dipped in tap water followed by three successive deionized water rinses, blot-dried on paper towels, and frozen at -10 °C for 72 h. Samples were freeze-dried for 72 h at -40 °C after which they were ground to a fine powder using a mortar and pestle.

Samples were analyzed for vitamin C content by titration according to the methods of the U.S. Pharmacopoeia (USP, 1980). One gram of sample was added to 50 mL of deionized water, filtered, and brought to a final volume of 100 mL with deionized water. A 20-mL aliquot was pipetted into a 250-mL conical flask and 150 mL of deionized water was added along with 1 mL of the starch solution (0.50 g of soluble starch in 50 mL of boiling deionized water). Samples were titrated with 0.005 mol·L⁻¹ of iodine solution to blue at the end point.

Total phenolics were determined using gallic acid according to the method of Slinkard et al. (1999). One-gram samples were placed in opaque bottles and 25 mL of 95% methanol was added, agitated for 30 min after which another 25 mL of methanol was added to each sample. Samples were filtered, brought up to a 50-mL volume with 95% methanol and thoroughly mixed. Approximately 20 μ L of each sample was pipetted into cuvettes, followed by 1.58 mL of deionized water, 100 μ L of Folin Coulteau, and 300 μ L of NaCO₃ and allowed to sit in the dark for 2 h. Samples were read using a Shimadzu ultraviolet-2401 PC ultraviolet-VIS Spectrophotometer (Shimadzu Corporation, New District Suzhou, China)

with absorbance measured at a wavelength of 765 nm.

The antioxidant capacity of the vegetable samples was determined based on DPPH following the methods of Chaires-Martinez et al. (2009) and Seal (2011). Samples (0.5 g) were combined with 50 mL of 95% methanol, shaken for 50 min, and filtered to a final volume of 50 mL with 95% methanol and thoroughly mixed. After mixing, 3.9 mL of DPPH solution and 100 μ L of the extracts were pipetted into cuvettes, allowed to sit in the dark for 30 min, and absorbance was measured at 515 nm using a Shimadzu ultraviolet-2401 PC ultraviolet-VIS Spectrophotometer (Shimadzu Corporation).

Beta-carotene content was determined using the method of Scott (2001) and saponification based on the method of Larsen and Christensen (2005). One gram of sample was added to 20 mL of 100% acetone and shaken for 20 min. The extractant was filtered and 50 mL of diethyl ether/petroleum ether stock (100 mL diethyl ether in 900 mL petroleum ether) and deionized water were added and allowed to precipitate. The precipitate was discarded and 0.05 g of butylated hydroxytoluene, 50 mL of KOH/methanol stock (100 g KOH in 1 L of 95% methanol) was added. This step was repeated three times using 300 mL of deionized water, after which 5 g of sodium sulfate was added to reduce moisture. The concentration of carotenoids was measured at 450 nm using a Shimadzu ultraviolet-1700, Pharmaspec Spectrophotometer.

Data were combined by treatments and tested by analysis of variance using the General Linear Model procedure (SAS Institute, 2007) with mean separation by Fisher's protected least significant difference at 0.05.

Results and Discussion

Analysis of variance for growth responses of the four vegetables is summarized in Table 1. Organic amendments did not significantly influence biomass production; however, species impacted fresh and dry biomass. There was no significant interaction between organic amendments and species; therefore, only the main effects are presented.

The main effect of fertilizer amendments on growth responses is shown in Table 2. Although the fertilizer amendments did not influence biomass production, there were trends toward greater fresh and dry biomass yield among plants receiving NPK or PL treatments. Overall, however, the two organic

Table 1. Statistical significance from analysis of variance of fertilizer amendments (ORAMD), species, and ORAMD $\times$ species for growth responses of Amaranth, Celosia, Gboma, and Long Bean.

		Fresh biomass			Dry biomass		
		Edible ^x	Inedible	Total	Edible	Inedible	Total
Source of Variation	df	(kg·ha ⁻¹)					
ORAMD ^y	2	NS	NS	NS	NS	NS	NS
Species	2	*** ^z	***	***	***	***	***
ORAMD × species	4	NS	NS	NS	NS	NS	NS

^zSignificant at $P = 0.0001$ (***); NS = nonsignificant.

^yORAMD = organic amendments (poultry litter, Megabloom—fish protein-based).

^xEdible (leaves for Amaranth, Gboma, and Celosia and pods for Long Bean); Inedible (lignified stems and roots at final harvest).

amendments were equally as effective as NPK in the trends toward higher production. The main effect of species on growth responses of fresh and dry biomass yield is shown in Table 3. Amaranth, Celosia, and Long Bean produced greater fresh and dry biomass than Gboma. However, Long Bean and Celosia produced greater inedible dry biomass compared with Amaranth and Gboma. Total dry biomass was greatest for Long Bean but similar to that produced by Amaranth and Celosia.

Because there was a significant interaction between organic amendment and species for antioxidant content, the effect of the interaction and not the main effects of amendment and species are presented (Table 4). Vitamin C content was highest among Amaranth and Celosia plants receiving NPK compared with the other two treatments, whereas that of Gboma was higher among plants receiving MB. Although β -carotene content was similar among Amaranth plants receiving both MB and PL and substantially greater than plants receiving NPK (Table 4), that of Celosia and Gboma was enhanced by MB and NPK fertilizer.

Total phenolic content was higher among Amaranth plants receiving NPK but was enhanced among Celosia and Gboma plants receiving PL (Table 4). Among Long Bean plants, however, total phenolic content was greater among plants receiving MB or NPK fertilizer. DPPH activity was enhanced by MB, MB and NPK, or MB only among Amaranth, Celosia, Gboma, and Long Bean plants, respectively.

These results show that species exerted a stronger influence on yield than organic amendments and among the leafy greens; Amaranth and Celosia produced a 39% greater fresh and dry biomass yield than did Gboma. Although organic amendments had no significant impact on biomass production, there were trends toward a positive response by the plants. For example, plants receiving PL produced 10% and those receiving MB a 23% greater fresh biomass yield than those receiving NPK. Although not measured in this study, nutrients in organic fertilizer are released through mineralization by soil microorganisms (Kelly and Boyhan, 2009). Depending on soil conditions such as pH and moisture content, mineralization rates can be impacted, and it is probable that the lack of response to organic amendments in this study could be related in part to mineralization rates resulting in fewer nutrients available for plant uptake (Boyhan and Kelly, 2010). Although we concentrated on the N (P and K) in the organic amendments, it is worthy of note that other essential nutrients are present. For example, Fulhage and Pfoest (2009) have reported the presence of other essential major and minor elements including sulfur, calcium, magnesium, manganese, copper, and zinc and estimated their availability ranged from 80% to 100% during the growing season after application.

In fact Whitmore (2007) reported that 40% of total N from composted PL was available during the first year and the remainder at the rate of 6% to 12% per year thereafter because

Table 2. Main effect of fertilizer amendments on growth responses of Amaranth, Celosia, Gboma, and Long Bean.^z

	Fresh biomass			Dry biomass		
	Edible ^y	Inedible	Total	Edible	Inedible	Total
ORAMD						
	(kg·ha ⁻¹)					
Megabloom ^x	565 a	796 a	1361 a	174 a	128 a	303 a
NPK	702 a	1049 a	1751 a	158 a	136 a	294 a
Poultry litter	805 a	1095 a	1907 a	178 a	166 a	345 a

^zMean separation within columns followed by the same letter are not significantly different based on least significant difference (0.05%).

^yEdible (leaves for Amaranth, Gboma, and Celosia and pods for Long Bean); Inedible (lignified stems and roots at final harvest).

^xORAMD = organic amendments (poultry litter, Megabloom—fish protein-based).

NPK = nitrogen–phosphorus–potassium.

Table 3. Main effect of species on growth responses of main effect of species on growth responses of Amaranth, Celosia, Gboma, and Long Bean.

Species	Fresh biomass			Dry biomass		
	Edible	Inedible	Total	Edible	Inedible	Total
	(kg·ha ⁻¹)					
Amaranth	887 a	1127 a	2015 a	193 a	164 b	357 ab
Celosia	814 a	1280 a	2094 a	264 a	192 a	455 a
Gboma	372 b	540 b	911 b	60 b	75 c	111 c
Longbean	1078 a	1177 a	2471 a	292 a	275 a	567 a

^zORAMD = organic amendments (poultry litter, Megabloom—fish protein-based).

^yMean separation within columns followed by the same letter are not significant based on least significant difference at the 5% level.

^xEdible (leaves for Amaranth, Gboma, and Celosia and pods for Long Bean); Inedible (lignified stems and roots at final harvest).

*Significant at $P = 0.01$, ** $P = 0.001$, *** $P = 0.0001$; NS = nonsignificant.

Table 4. Effect of interaction between species and organic fertilizer amendments on antioxidant content and capacity of Amaranth, Celosia, Gboma, and Long Bean.

ORAMD ^z	Vitamin C	β -carotene (mg/100 g)	Total phenolics	Antioxidant capacity ^y (μ mol AAE/g)
	<i>Amaranth</i>			
Megabloom	156 b	91.6 a	467 b	30.6 a
NPK	188 a	77.9 b	542 a	25.3 b
Poultry litter	133 b	93.5 a	362 c	24.5 b
Significance	**	*	**	*
	<i>Celosia</i>			
Megabloom	163 b	81.1 a	400 b	30.7 a
NPK	197 a	87.9 a	420 ab	31.8 a
Poultry litter	185 ab	54.2 b	440 a	19.8 b
Significance	*	*	*	*
	<i>Gboma</i>			
Megabloom	160 a	189.4 ab	200 b	77.2 a
NPK	102 b	200.2 a	304 ab	68.6 a
Poultry litter	102 b	157.8 b	453 a	57.2 b
Significance	*	*	*	*
	<i>Longbean</i>			
Megabloom	—	—	556 a	47.1 a
NPK	—	—	592 a	37.1 b
Poultry litter	—	—	401 b	39.8 b
Significance	—	—	*	*

^zORAMD = organic amendments (poultry litter, Megabloom—fish protein-based).

^y2,2-Diphenyl-1-picrylhydrazyl (DPPH) percent radical scavenging quenched.

*Significant at $P = 0.05$ and *** $P = 0.001$. Mean separation within columns followed by the same letter are not significant based on least square means at the 5% level.

NPK = nitrogen–phosphorus–potassium.

of slow mineralization rates, which proves the findings of researchers in Georgia and Florida that are suggesting an advantage of applying 50% more organic fertilizer 14 to 20 d earlier than normal to compensate for these slow rates. These results suggest that plant responses would be more positive in subsequent years.

The antioxidant and total phenolics content as well as DPPH activity of the vegetables varied with species. For example, NPK enhanced vitamin C content and total phenolics in Amaranth but not β -carotene or DPPH activity. These values for vitamin C content exceeded the 80 to 100 mg/100 g of the

recommended daily values for an adult male (Babalola et al., 2010). These results are inconsistent with those of Hornick (1989) with kale and hibiscus (Muso and Ogaddiyo, 2012) who reported lower vitamin C content with increased N fertilization. The major pathway of vitamin C synthesis in plants is through L-galactose mediated by the enzyme L-galactose dehydrogenase (Wheeler et al., 1998). Therefore, the increase in vitamin C content in this study could be attributable in part to a decrease in protein production and an increase in carbohydrate production (Muso and Ogaddiyo, 2012). High vitamin C content in the leaves may make plants more tolerant of stress because reducing vitamin C content increases susceptibility to stresses (Conklin, 2000).

There were also species differences in β -carotene content, although generally it appears that all three amendments enhanced β -carotene content. For example, β -carotene levels were similar among Amaranth plants receiving MB and PL, whereas it was enhanced by MB and NPK in Celosia and Gboma. Research has shown that light enhances the biosynthesis of phenolics in the chloroplasts of the cells and thus tends to accumulate in high amounts in the vacuoles or deposits in secondary cell walls as lignin (Kefeli et al., 2003). One of the more important findings of this study is the impact of MB on increasing antioxidant capacity of all four species and in all cases equaling or exceeding the standard values for leafy vegetables (Tarwadi and Agte, 2003).

The analysis of DPPH ability showed that Gboma and Longbean had a relatively high antioxidant potential as evidenced by the reduction of the DPPH, radicals manifested by the decrease in absorbance at 516 nm. When DPPH accepts an ion donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. It has been determined that the antioxidant effect of plant products is mainly the result of radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes (Rahman and Moon, 2007). Furthermore, the antioxidant activity of the phenolic compounds is mainly the result of their redox properties, which can play an important role in adsorbing and neutralizing free radicals and quenching the singlet and triplet oxygen (Hasan et al., 2009). Although the values for DPPH activity were greater among plants receiving MB in particular, values for all species in general were consistent with the findings of others (Ferreira et al., 2005). This suggests that these plants have the ability to scavenge significant quantities of free radicals when consumed in proper amounts.

Overall, these results show that there were no significant impact of MB or PL on biomass production, but based on the results of the nutritional analysis, it appeared that main impact of the organic amendments were in high content of total phenolics, antioxidants, and DPPH activity.

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