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Effects of Vermicompost, Tithonia Green Manure and Urea on Quality of Swiss Chard (*Beta Vulgaris* L. Var. *Cicla* L.) in Kenya

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Abstract

Swiss chard (*Beta vulgaris* L. var. *cicla*) is a popular vegetable because it is nutritious, robust, easy to grow. It requires regular applications of nitrogen, which causes accumulation of oxalates and nitrates within the plant. Oxalates and nitrates are known health hazards but the use of organic fertilizers have been shown to reduce their accumulation and to promote accumulation of beneficial phytochemicals. We therefore determined the effect vermicompost (VC), *Tithonia diversifolia* green manure (Tithonia) and urea on the quality of Swiss chard. The experiment was laid out in a Randomised Complete Block Design (RCBD) replicated three times with thirteen treatments. Leaf nutrient elements, TSS, polyohenols, Vitamin C and antioxidants were determined using standard procedures. Treatment effects were significant only for aluminum, phosphorus and calcium. The values were highest on VC 50 kg N ha^{-1} and Urea 50 kg N ha^{-1}, Tithonia 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}, and VC 50 kg N ha^{-1}, and VC 100 kg N ha^{-1} for aluminum, phosphorus and calcium, respectively. Lowest response was reported on Tithonia 50 kg N ha^{-1}, control and Tithonia 50 kg N ha^{-1} + Urea 50 kg N ha^{-1} for aluminum, phosphorus and calcium, respectively. The treatments significantly influenced the percentage of polyphenols, vitamin C and the antioxidant capacity while no significance was reported for total soluble solutes. The polyphenols and vitamin C content were highest on Tithonia 100 kg N ha^{-1} and control, respectively. Low polyphenol and vitamin C contents were recorded on VC 50 kg N ha^{-1} and VC 100 kg N ha^{-1}, respectively. A similar trend was observed for antioxidant activity since a positive correlation was observed between the polyphenols and vitamin C and the antioxidant activity. Tithonia green manure improves Swiss chard quality.

Keywords: Swiss chard, vermicompost, tithonia, Urea, polyphenols, vitamin C

1. Introduction

Swiss chard (*Beta vulgaris* L. var. *cicla* L.) a member of the family Chenopodiaceae is a biennial plant (Kretschmer, 1999). It is among the nitrate accumulating vegetables (Santamaria, 2006) because it is one of the species known to "luxury-consume" the NO_3^- ion and accumulate it to high levels in particular vacuoles to the point that such levels are often considered a health hazard for human consumption.

Swiss chard is a very important crop from the nutritional point of view (Platt, 1962). It is highly valued because it is available all year round and for its nutritional properties. It is an excellent source of vitamins K and A, folic acid, dietary fibre and protein, very good source of vitamins C and E, most B vitamins and numerous minerals, especially calcium, potassium, phosphorus and manganese, as well as respectable amounts of niacin, and folic acid (Smatanova, Richter & Hlusek, 2004; Leskovar & Piccinni, 2005; Citak & Sonmez, 2009). It is very low in saturated fat and cholesterol but it is naturally quite high in sodium (Mitic, Jovanovic, Dimitrijevic, Cvetkovic & Stojanovic, 2013). The species is also concentrated in health-promoting phytonutrients such as carotenoids and flavonoids which provide powerful antioxidant protection (Bimova & Pokluda, 2009; Fairman, 2011). Many of these vitamins and nutrients help fight various types of cancer and promote good cardiovascular health (Masarirambi, Mbokazi, Wahome & Oseni, 2012).

On the other hand, Swiss chard is associated with antinutrients such as oxalates and nitrates (Santamaria, Elia, Serio & Todaro, 1999) which accumulate to high levels during growth. Excessive levels of nitrates and oxalates have detrimental effects to human health (Santamaria et al., 1999; Ikemoto, Teraguchi & Kogayashi, 2002; Ishiwata et al., 2002).

Some relationships between nitrates, ascorbic acid and oxalates have been established. There is an inverse relationship between ascorbic acid and nitrate content in leaf tissues (Alderfasi, Moftah & Aljuaed, 2010; Citak & Sonmez, 2010; Mozafar, 1993) and a slight positive correlation between nitrates and oxalates (Koh, Charoenprasert & Mitchell, 2012; Elia, Santamaria & Serio, 1998). This means that the nitrate and oxalates can be reduced while increasing the ascorbic acid in leafy vegetables like Swiss chard.

The relationship between nitrate accumulation and ascorbic acid suppression could be changed by using organic sources alone or in conjunction with lower amounts of N from inorganic sources (Alderfasi et al., 2010; Citak & Sonmez, 2010; Mozafar, 1996; Muramoto, 1999. Vermicompost (VC) and tithonia are potential candidates of the organic sources that could be used to improve the quality of Swiss chard. Tithonia is a plant that is abundant on hedges of farms and roadsides in western Kenya and is known as an effective source of N for crops (Jama, Palm & Buresh, 2000).

Vermicomposting is a waste management technology that involves decomposition of organic fraction of solid waste in an ecofriendly way to a level in which it can be easily stored, handled and applied to agricultural fields without any adverse effects (Singh, Pathak, A.K. Verma, V.K., Verma & Deka, 2011; Khwairakpam & Bhargara, 2009; Aira, Monroy & Dominguez, 2009). The product of vermicomposting is a finely divided, peat-like material with a low C:N ratio, excellent structure, porosity, aeration, drainage and moisture holding capacity, and it supplies a suitable mineral balance, improves nutrient availability and could act as complex-nutrient source granules (Dominguez & Edwards, 2011).

2. Materials and Methods

2.1 Site Description

The study was conducted between February 2016 and February, 2017 at the University of Kabianga farm in Kericho County, Kenya. The site lies at an altitude of 2163 M above sea level (0^o 49’0 N, 35^o 49’60 E). The average rainfall is 900-1200 mm per annum distributed mainly between the months of March and December with two distinct peaks in May and October. The soils at the site are Nitisols which are acidic, moderately deep and well drained (Jaetzold & Schmidt, 1982). The soil physical properties of the experimental site were analysed before the start of the experiments. Results were as follows; % sand = 23.07, % clay = 58.20 and % silt = 18.73 giving the grade of the soil as clay. Selected soil chemical properties at the site were as listed in table 1, while selected chemical properties for the organic fertilizers used in the study are presented on table 2.

Table 1. Selected chemical properties of the experimental site soil

pH	N ppm	P ppm	K ppm	Ca ppm	Mg ppm	Mn ppm	Zn ppm	Cu ppm	Fe ppm	Al ppm
4.64	75	116	574	577	122	157	17	17	51	813

Table 2. Vermicompost analysis

	%N	%P_2O_5	%K_2O	%CaO	%MgO	%S	Ca ppm	Pb ppm	Zn ppm	Cu ppm	Fe ppm	S%	CO-3
Vermicompost	4.20	4.02	0.68	9.42	0.73	0.48	43.00	48.00	110.00	51.00	2320.00	0.60	5.00

2.2 Experimental Design and Crop Management

The experimental plots were ploughed, and harrowed. The experiment was laid out in a Randomised Complete Block Design (RCBD) replicated three times with 13 treatments as listed below:

1. Control
2. Vermicompost (50 kg N ha^{-1})
3. Vermicompost (100 kg N ha^{-1})
4. Tithonia (50 kg N ha^{-1})
5. Tithonia (100 kg N ha^{-1})
6. Urea (50 kg N ha^{-1})
7. Urea (100 kg N ha^{-1})

8. Vermicompost (25 kg N ha^{-1}) + Urea (25 kg N ha^{-1})
9. Vermicompost (50 kg N ha^{-1}) + Urea (50 kg N ha^{-1})
10. Tithonia (25 kg N ha^{-1}) + Urea (25 kg N ha^{-1})
11. Tithonia (50 kg N ha^{-1}) + Urea (50 kg N ha^{-1})
12. Vermicompost (100 kg N ha^{-1}) without Triple Super Phosphate (TSP)
13. Tithonia (100 kg N ha^{-1}) without TSP

All the treatments, except 1, 12 and 13, had the recommended amounts of P (30 kg ha^{-1}) applied.

Vermicompost was obtained from composting waste tea leaves and poultry manure using *Eisenia foetida* [which is the most commonly used species (Haimi & Huhta, 1990) and is raised in Kenya by several flower farms from which the worms were sourced. A bed was prepared with a plastic sheet bottom and 30 cm wooden walls. A layer of 15 cm waste tea leaves were placed on the bed and poultry manure was added at the weight ratio of 3:1 (i.e waste tea leaves: poultry manure). The mixture was then moistened before addition of the worms by placing small handfuls of material rich in the worms into 'holes' of about 50 cm apart. The bed was then covered using a dark polyethene plastic and allowed to mature for 60 days during which time the materials were watered and turned once every week for the first two weeks and fortnightly thereafter until maturity. Occasionally, additional poultry manure was buried at different parts of the bed as extra food for the worms. There were earthworms harvested from the mature vermicompost to allow for the use of the vermicompost. The vermicompost was air dried for seven days before field application. On the other hand, Tithonia was sourced from neighbouring farms and roadside. The green leaf biomass of Tithonia has been shown to be high in nutrients averaging about 3.5 % N, 0.37% P and 4.1 % K on a dry matter basis (Jama et al., 2000). The vegetative parts (tender shoots and leaves) were chopped into small pieces and used as green manure.

The size of experimental plot was 2.1 m x 2.1 m. Plant establishment was done using nursery grown one month-old seedlings of Swiss chard; variety 'Giant Fordhork' at a spacing of 30 cm x 15 cm. The VC, Tithonia and half the amount urea were worked into the soil just before transplanting was done with the remaining half of the urea applied four weeks after transplanting (for the treatments where urea was used as a sole source of N). Where Urea was to be used in combination with the organic source of N, all the urea was applied as a top-dress. All the TSP was applied at planting. All recommended cultural practices were adopted uniformly according to standard crop requirements.

Ten inner rows plants/plot were tagged and used for data collection. Data on nutrient elements, polyphenols, total soluble solutes, vitamin C and antioxidant activity was taken based on the tagged plants.

2.3 Determination of Leaf Nutrient Elements

Leaf samples were oven-dried at 65 ^{o}C for 3 days and ground into powder, they were prepared and analysed for Calcium, Magnesium, Copper, Zinc, Iron, Manganese using atomic absorption spectrophotometry on a dry weight (DW) bases according to Zarei (1995). Phosphorus (P) was determined using Olsen method and Potassium (K) using flame photometer (Humphries, 1956).

2.4 Determination of Total Polyphenols

Extraction of total polyphenols was done according to the procedure by Karori, Wachira, Wanyoko & Ngure (2007). Ground Swiss chard samples (0.2 g) were weighed into graduated extraction tubes and 5 ml of 70 % hot methanol/water v/v (MeOH) added, stoppered and mixed under vortex. Incubation followed at 70 ^{o}C for 10 minutes (water bath) with vortexing at 0, 5, and 10 minutes. Cooling to rtp and then centrifuging at 3000 rpm for 10 minutes was done. A second extraction was performed and 10 ml with cold methanol/water v/v (MeOH) at 70%. Extracted samples were complexed using 75 % sodium carbonate and 10% Folin-ciocalteu reagent (Phenol) and absorbance read at 765 nm. The total polyphenol content was expressed as a percentage by mass on a sample dry matter (Karori et al., 2007)

2.5 Determination of Vitamin C

Vitamin C (ascorbic acid) was extracted by blending 20 g of chopped fruits with 100 mL of 0.4% (w/v) oxalic acid solution and quantified using the potassium ferriccyanide method (Hashmi, 1973).

2.6 Determination of Antioxidant Activity

The total reducing power of Swiss chard sample was determined according to the Ferric Reducing Antioxidant Power (FRAP) method as described by (Yildirim, Mavi, Kara, 2001). A 2.5 mL volume of the Swiss chard sample was mixed with 2.5 mL phosphate buffer solution (0.2 M, pH = 6.6) and 2.5 mL of 1 % potassium ferriccyanide [$K_3Fe(CN)_6$] in tubes. The mixture was placed in a water bath of 50 °C, for 20 minutes. A volume of 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture and mixed thoroughly. A volume of 2.5 of this mixture was then mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ of 0.1% solution and allowed to stand for 10 minutes. The absorbance of the mixture was measured at 700nm using a UV-VIS spectrophotometer; the higher the absorbency of the reaction mixture, the greater the reducing power. Ascorbic acid was used as a positive control for this assay. All procedures were performed in triplicate.

2.7 Data Analysis

The data was subjected to the Analysis of Variance (ANOVA) using Mstat C computer software package (Russel, 1995). The Least Significant Difference (LSD) procedure was then used to separate differences among the treatment means at $p < 0.05$.

3. Results and Discussion

Nutritional quality of Swiss chard was characterized by total soluble solids (TSS), ascorbic acid, antioxidant capacity, total polyphenol content, total soluble solutes and minerals (nitrogen, phosphorus, potassium, calcium, magnesium, manganese aluminum, copper, iron and zinc).

3.1 Mineral Elements

Table 3. Effects of Vermicompost, Tithonia and Urea on Leaf Tissue Analysis (Season 1)

Treatments/ nutrients	% Al	% Ca	% K	% Mg	% Mn	% N	% P	Cu (ppm)	Fe (ppm)	Zn (ppm)
1. Control	0.48	1.36	4.47	0.92	0.07	2.75	0.44	29.33	147.30	59.30
2. VC50 kg N ha^{-1}	0.41	0.90	3.42	0.56	0.07	2.42	0.48	29.33	112.36	59.00
3. VC100 kg N ha^{-1}	0.40	1.03	3.94	0.66	0.07	2.71	0.46	29.33	135.70	70.30
4. Tithonia 50 kg N ha^{-1}	0.30	0.58	3.13	0.46	0.04	3.88	0.54	29.00	114.7	63.00
5. Tithonia 100 kg N ha^{-1}	0.31	0.85	4.13	0.79	0.34	2.66	0.43	29.33	84.30	57.70
6. Urea 50 kg N ha^{-1}	0.46	1.25	4.61	0.80	0.07	3.59	0.53	29.33	187.30	42.10
7. Urea 100 kg N ha^{-1}	0.36	1.03	3.58	0.74	0.07	2.93	0.51	29.67	126.00	58.30
8. VC25 kg N ha^{-1}+Urea 25 kg N ha^{-1}	0.41	0.97	4.61	0.82	0.07	4.10	0.60	30.33	145.30	61.30
9. VC50 kg N ha^{-1} + Urea 50 kg N ha^{-1}	0.55	1.03	3.92	0.76	0.07	2.54	0.47	29.67	149.30	63.00
10. Tithonia 25 kg N ha^{-1}+ Urea 25 kg N ha^{-1}	0.45	1.11	4.57	0.71	0.08	2.74	0.45	30.00	174.00	62.70
11. Tithonia 50 kg N ha^{-1}+ Urea 50 kg N ha^{-1}	0.41	0.86	3.82	0.65	0.06	2.91	0.52	29.67	126.70	66.00
12. VC100 kg N ha^{-1}	0.40	0.95	3.51	0.65	0.07	2.56	0.50	32.33	98.00	64.00
13. Tithonia 100 kg N ha^{-1}	0.35	1.10	4.13	0.76	0.07	2.83	0.45	31.33	88.70	65.70
Significance	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSD	0.14	0.59	1.19	0.35	0.22	1.28	0.12	2.11	67.02	19.32

Table 4. Effects of Vermicompost, Tithonia and Urea on Leaf Tissue Analysis (Season 2)

Treatments/ nutrients	% Al	% Ca	% K	% Mg	% Mn	% N	% P	Cu (ppm)	Fe (ppm)	Zn (ppm)
1. Control	0.27	1.36	4.51	0.823	0.072	2.33	0.41	29.33	95.00	66.00
2. VC50 kg N ha^{-1}	0.22	1.05	4.81	0.80	0.07	3.35	0.53	28.33	80.70	65.00
3. VC100 kg N ha^{-1}	0.21	1.16	4.52	0.85	0.08	2.74	0.49	30.00	90.30	69.70
4. Tithonia 50 kg N ha^{-1}	0.21	1.19	4.72	0.88	0.08	2.74	0.47	29.00	91.70	66.30
5. Tithonia 100 kg N ha^{-1}	0.21	1.14	4.38	0.84	0.08	2.77	0.46	31.00	87.30	67.30
6. Urea 50 kg N ha^{-1}	0.21	1.23	4.73	0.89	0.09	2.88	0.48	29.00	93.70	69.70
7. Urea 100 kg N ha^{-1}	0.20	1.34	4.39	0.92	0.09	3.05	0.48	30.33	81.00	68.70
8. VC 25 kg N ha^{-1} +Urea 25 kg N ha^{-1}	0.21	1.32	4.68	0.75	0.09	2.57	0.45	28.33	85.70	67.30
9. VC 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}	0.21	1.11	4.27	0.78	0.07	2.60	0.45	28.67	72.70	68.30
10. Tithonia 25 kg N ha^{-1} + Urea 25 kg N ha^{-1}	0.20	1.09	4.89	0.90	0.09	2.52	0.47	29.33	94.30	66.70
11. Tithonia 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}	0.22	1.05	4.45	0.89	0.07	3.09	0.53	29.67	91.00	68.30
12. VC 100 kg N ha^{-1}	0.21	1.69	4.72	0.94	0.09	2.58	0.43	31.33	75.70	71.30
13. Tithonia 100 kg N ha^{-1}	0.23	1.50	5.60	1.00	0.09	2.65	0.42	30.33	93.00	67.70
Significance	NS	S	NS	NS	NS	NS	S	NS	NS	NS
LSD	0.08	0.18	1.21	0.27	0.02	0.71	0.07	2.61	26.04	15.70

For Aluminum, (Season 1) the highest levels (0.55%) were recorded in plants grown on soil that received VC at 50 kg N ha^{-1} and Urea at 50 kg N ha^{-1}. The least (0.30) was recorded on Tithonia at 50 kg N ha^{-1} with TSP application (Table 3).

Leaf tissue nitrogen showed inconsistent and non-significant response to treatments in both seasons (Tables 3 & 4). During the first season, the N content was highest (4.10%) on VC 25 kg N ha^{-1} + Urea 25 kg N ha^{-1} making a total of 50 kg N ha^{-1} and the least (2.42%) on VC 50 kg N ha^{-1}. In the second season, the highest N content was recorded on VC 50 kg N ha^{-1} (2.42%) was recorded on VC 50 kg N ha^{-1} and the least (2.33%) on the control where no form of fertilizer was applied. It is worth noting that though not significantly different, leaf N content was highest in vermicompost alone or combined with urea at the lowest rate of application (50 kg N ha^{-1}). This is contrary to findings by Hernandez et al. (2010) who observed that leaf N concentration in inorganic fertilizer treated plants was higher than organically treated plants. This is so because organic fertilizers are slow release N fertilizers and only mineralizes a fraction of total N estimated at 2% in a crop cycle (Castellanos, Uralle-Bueno & Aguilar-Santelises, 2000; Sikora & Szmidt, 2005) while urea is characterized as an N-rich source (Arroyo, Rojas & Rosales, 2003) with high solubility of N (Capulin, Nunez, Etchevers & Baca, 2001). All in all, the fertilizer treatments afforded sufficient levels of N (2.33-4.10 %) with reference to sufficiency levels of 2.50-4.50 % as given by Munson (1998).

Phosphorus content in Swiss chard leaves was significantly influenced in season 2 but not in season 1 (Tables 3 & 4). Two treatments yielded the highest content (0.5267 %) of tissue phosphorus; Tithonia at 50 kg N ha^{-1} + Urea at 50 kg N ha^{-1} and VC at kg N ha^{-1} during season 2. The lowest amounts (0.41 %) were observed on the control where no form of fertilizer was applied. These findings partly agree with those of Hernandez et al. (2010) who report no significant differences in P content in spinach leaf tissue. The levels of P (0.41-0.60 %) in the current study attained the sufficiency level for this element according to Munson, 1998 who provided a range of 0.20-0.75 %. It has been reported that green biomass of Tithonia is rapid and that Tithonia can supply plant available P at least as effectively as an equivalent amount of P from soluble fertilizer. Nziguheba, Palm, Buresha & Smithson (1998) reported that labile inorganic soil P as determined by extraction with anion exchange resin, was higher at 2 weeks after incorporation of 15 kg P-ha^{-1} as Tithonia (8.1 mg Pkg^{-1}) than as TSP (3.6 mg Pkg^{-1}) on acid soil. This could explain why it was possible to register a significantly higher P content in leaf tissues of Swiss chard grown on soil fertilized with Tithonia at 50 kg N ha^{-1} in addition to Urea at the same rate.

The response for potassium (K) was not significant in all the treatments across the seasons (Tables 3 & 4). However, K leaf tissue concentrations were highest (4.61 %) on Urea 50 kg N ha^{-1} as well as on VC 25 kg N ha^{-1} + Urea 25 kg N ha^{-1} and lowest (3.13) on Urea 25 kg N ha^{-1} during the first season. In season 2, the highest (5.60%) was recorded on 100 kg N ha^{-1} applied as Tithonia with no other fertilizer and the least on 100 kg N ha^{-1} applied as VC without any fertilizer. This is in agreement with Hernandez et al. (2010) who showed that leaf K content in plants treated with urea was higher than that of vermicompost though the differences were not significant. All the treatments presented K at sufficient levels (3.13- 5.6 %) based on sufficiency amounts (1.50-5.50 %) given by Munson (1998). Despite the highest level exceeding the limits by Munson (1998), K content is not high enough to cause toxicity set at > 6.00% (Munson, 1998). This findings emphasize the importance of urea, vermicompost and Tithonia as potential sources of K for Swiss chard production.

Calcium accumulation in the Swiss chard leaves was significantly influenced by the treatments in Season 2 but not in Season 1 (Tables 3 & 4). During season 1, the highest percentage (1.36%) was recorded on plants that received 0 kg N ha^{-1} and the least (0.58 %) on plants receiving 50 kg N ha^{-1} applied as green manure of Tithonia. In the second season, greatest (1.69 %) calcium accumulated on 100 kg N ha^{-1} supplied by vermicompost without any fertilizer application and the least (1.05 %) on plots that received 50 kg N ha^{-1} from urea and 50 kg N ha^{-1} from Tithonia. This means that sole organic fertilizer supplied more calcium to the plant than with combined sources i.e organic and inorganic fertilizer used in combination. The amount (0.58- 1.68%) of calcium across the treatments reached the sufficiency levels described by Munson (1998) as 1.00-4.00 %.

In season 1, magnesium content in the leaves was highest (0.92 %) on the control that received no fertilizer application and the least (0.46 %) was on Tithonia at 50 kg N ha^{-1} (Table 3). In season 2 (Table 4), the highest amounts (1.00 %) was recorded on Tithonia at 100 kg N ha^{-1} and least (0.75 %) on VC 25 kg N ha^{-1} + Urea 25 kg N ha^{-1}. However, magnesium content was not significantly influenced by the treatments in both seasons. The amount of magnesium (0.46-1.00 %) across the treatments fitted within the sufficiency levels (0.25-1.00 %) and below the toxicity levels of >1.50% (Munson, 1998).

During the first season (Table 3), Mn concentration was highest (0.34 %) on Tithonia at 100 kg N ha^{-1} and lowest (0.04 %) on Tithonia at 50 kg N ha^{-1}. In season 2 (Table 4), the highest (0.09 %) was recorded on Tithonia applied at the rate of 100 kg N ha^{-1} and the least on VC 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}.

Copper leaf content was highest (32.33 ppm) on VC 100 kg N ha^{-1} and least (29.00 ppm) on Tithonia at the rate of 50 kg N ha^{-1} while in the second season, the highest (31.33 ppm) was recorded on VC 100 kg N ha^{-1} without any inorganic fertilizer application and the least (29.00 ppm) on VC 50 kg N ha^{-1} and VC 25 kg N ha^{-1} + Urea 25 kg N ha^{-1}. The treatment effects were however not significantly different in both seasons (Tables 3 & 4).

The response of leaf iron content was not significantly influenced by the treatments in both seasons (Tables 3 & 4). However, during the first season, the highest Fe content (187.30 ppm) was recorded on Urea 50 kg N ha^{-1} and the least (88.70 ppm) on Tithonia at kg N ha^{-1} without any inorganic fertilizer application. In the second season, the control contributed the highest (95.00 ppm) and VC 50 kg N ha^{-1} + Urea 50 kg N ha^{-1} yielded the least (72.70 ppm).

Zinc concentration in season 1 (Table 3) was highest (70.30 ppm) on VC 100 kg N ha^{-1} and least on Urea 50 kg N ha^{-1}. During the second season (Table 4), it was highest (71.30 ppm) on VC 100 kg N ha^{-1} without any inorganic fertilizer and least (65.00 ppm) on VC 50 kg N ha^{-1} with an application of TSP.

Table 5. Effects of Vermicompost, Tithonia and Urea on Selected Quality Parameters

Treatments	Polyphenols (%)	Total Soluble Solutes (^{o} Brix)	Vitamin C (%)	Antioxidant (%)
1. Control	1.34	0.035	32.46	12.14
2. VC 50 kg N ha^{-1}	0.47	0.05	15.68	6.75
3. VC 100 kg N ha^{-1}	0.64	0.03	21.36	8.70
4. Tithonia 50 kg N ha^{-1}	0.95	0.03	22.46	10.12
5. Tithonia kg N ha^{-1}	1.197	0.04	23.06	10.28
6. Urea 50 kg N ha^{-1}	0.82	0.04	14.56	4.87
7. Urea 100 kg N ha^{-1}	0.90	0.03	21.17	10.38
8. VC 25 kg N ha^{-1} +Urea 25 kg N ha^{-1}	0.98	0.04	26.51	10.11
9. VC 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}	1.23	0.03	25.73	9.84
10. Tithonia kg N ha^{-1} + Urea 25 kg N ha^{-1}	1.13	0.04	21.85	10.74
11. Tithonia 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}	0.70	0.04	15.79	6.13
12. VC 100 kg N ha^{-1}	0.84	0.03	13.88	6.72
13. Tithonia 100 kg N ha^{-1}	1.64	0.03	31.58	11.58
Significance	S	NS	S	S
LSD VALUE	0.61	0.02	1.50	3.41

3.2 Polyphenols

The percent polyphenols were significantly influenced by the fertilizer treatments (Table 5). The percent polyphenols in the leaves of Swiss chard were highest (1.64 %) on plants that were fertilized with Tithonia at 100 kg N ha^{-1} without any inorganic fertilizer. This treatment was not significantly different with the amount of polyphenols recorded on plants that received no fertilizer application (control). The lowest amounts of polyphenols (0.47 %) on vermicompost at 50 kg N ha^{-1}. These findings are similar to those reported by Lesser and Treutter (2005) who indicated that lower levels of phenolic and flavonoid compounds in plants grown under high N supply. In Contrast, other researchers have reported that higher phenolic contents are observed when nutrient availability was limited at the lowest applied nitrogen treatment (Ibrahim, Jaafar, Rahmat & Rahman, 2011; Munene, Changamu, Korir & Gweyi-Onyango, 2017; Salahas, Papasavvas, Giannakopoulos, Tselios & Savvas, 2011; Argyropoulou, Salahas, Hela & Papasavvas, 2015). This could be an indication that biosynthesis of secondary plant metabolites is stimulated by N deficiency as reported by Scheible et al. (2004). To account for the findings in the current research, the results of high percentage of polyphenols on the plants fertilized with Tithonia without application of any inorganic fertilizer could be as a result of the fast decomposition of Tithonia leaf biomass suggesting a more rapid release of N than uptake of N by the plants leading to a low crop recovery of the N in the Tithonia as was observed by (Jama et al., 2000) and hence the high amounts of polyphenols. Furthermore, Tithonia has been reported to have a high N content but it is too low in phosphorus to meet the crop P demand (Palm, Gachengo, Delve, Cadisch & Giller, 2001) resulting in a P- nutrient stress that could have caused an increase in the synthesis and accumulation of the polyphenols in the Swiss chard plants in this treatment. Vermicompost fertilization has been reported not exerting any significant effects in the expression of bioactive compounds (Yusof, Ramasamy, Mahmoud & Yaacob, 2018) as was observed in the current study. Coria-Cayupan, De Pinto and Nazareno, (2009); Pant, Radovich, Hue, Talcott & Krenek (2009) and Lujan-Hidalgo et al. (2015) reported low secondary metabolites with application of vermicompost. This observation can be attributed to the fact that vermicompost provides nutrients to the plants in readily available forms such as nitrogen and enhances plant nutrient uptake (Adhikary, 2012). This is evident in the current study as the major nutrients (N. P and K) were highest in leaves of Swiss chard fertilized with VC at various levels (sole or in combination with inorganic fertilizer) (Tables 3 and 4).

3.3 Total Soluble Solutes

The greatest TSS (0.05) were recorded on VC 50 kg N ha^{-1} and least on urea at 100 kg N ha^{-1}. However, the differences were not significantly different among the treatments. Different observations were made by Fatondji, Pasternak & Woltering, 2008; Massri & Labban, 2014; Vazquez et al., 2018). They reported that organic fertilizers are not related to total soluble solids.

3.4 Vitamin C

Vitamin C is one of the most important components in plants and animals with important antioxidant and metabolic functions in the form of vitamin C but humans have lost the capacity to synthesize it (Nishikimi, Fukuyama, Minoshima, Shimizu & Yagi, 1994) and hence it must be provided from the diet (Hillstrom, Yacapin-Ammons & Lynch, 2003).

The amount of vitamin C in Swiss chard was significantly affected by the treatments. More vitamin C accumulated in the plants grown with no form of fertilizer and least on VC 100 kg N ha^{-1} without any other inorganic fertilizer application. However, Tithonia organic fertilizer yielded almost the same as the control and much more than that accumulated on plants grown on VC 100 kg N ha^{-1} without any other fertilizer.

There are varied observations from literature regarding the effect of N on the accumulation of vitamin C in plants. Some researchers have reported that increasing doses of nitrogen depressed vitamin C content (Dzida, Jarosz & Michalojc, 2012; Rajasree & Pillai, 2012; Stefanelli, Goodwin & Jones, 2010; Welch, 2016). Contrary, Miceli & Miceli (2014); Hassan, Mijin, Yuseff, Ding & Wahab (2012) and Ivanovic. (2018) reported that increasing the rate of N fertilization increased the content of vitamin C. This difference could be explained by the differences in plant species, climatic conditions, fertilization and other agricultural practices used (Ivanovic et al., 2018).

The current study clearly indicates that different types of fertilizer have different effects on vitamin C. Vermicompost at its highest rate of application without inorganic fertilizer yielded the least vitamin C which could be linked to the fact that vermicomposts have been shown to have low levels of ammonium-N and higher Nitrate-N (Edwards & Burrows, 1988; Arancon & Edwards, 2004; Arancon, Edwards, Bierman, Welch & Metzger, 2004) which could have been the reason why VC yielded low content of vitamin C since plants supplied with ammonium-N fertilizer contain less ascorbic acid than the same plants supplied with nitrate-N (Mozafar, 1993).

3.5 Antioxidant Activity

The antioxidant activity of Swiss chard was significantly influenced by the treatments. The highest activity (12.14%) was recorded on the control and the least (4.87%) on urea at 50 kg N ha^{-1}. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu, Mazza, Gao & Oomah, 1998). The antioxidant activity of plant parts is mainly contributed by the constituent phenolic and flavonoid compounds (Soobrattee, Neergheen, Luximon-Ramma, Aruoma & Bahorun, 2005; Panche, Diwan & Chandra, 2016). Nitrogen fertilization has been shown to affect antioxidant activity in basil treated with the highest applied N level exhibiting lower antioxidant activity (Nguyen & Niemeyer, 2008).

3.6 Relationship between Antioxidant Activity and TSS, Vitamin C and Polyphenols

Polyphenols positively correlated with Vitamin C and antioxidant activity while total soluble solids negatively correlated with the vitamin C, polyphenols and antioxidant activity in line with the carbon/Nutrient balance theory and growth rate and growth-differentiation balance hypothesis, indicating that the allocation of plant metabolism toward higher carbon-containing components (ascorbic acid and polyphenols) and lower nitrogen-containing compounds (TSS). Total phenols are effective antioxidants or free radical scavengers (Ogembo, 2015; Okello, 2015) in leafy vegetables. Similarly, Vitamin C has been known to have marked nucleophilic properties and the ability to capture and deactivate free radicals or reactive oxygen species produced by metals (Jiraungkoorskul & Sahaphong, 2007). This is a clear indication of the positive relationship between polyphenols and the antioxidant activity of Swiss chard.

Table 6. Coefficients of Correlation (R) Between Antioxidant Activity and TSS, Vitamin C and Polyphenols

Quality parameters	R values
Total Soluble Solutes (°Brix)	-0.474
Vitamin C (%)	+0.898
Polyphenols (%)	+0.748

4. Conclusion & Recommendations

Treatment effects were significant only for aluminum, phosphorus and calcium. The values were highest on VC 50 kg N ha^{-1} and Urea 50 kg N ha^{-1}, Tithonia 50 kg N ha^{-1} + Urea 50 kg N ha^{-1}, and VC 50 kg N ha^{-1}, and VC 100 kg N ha^{-1} for aluminum, phosphorus and calcium, respectively. On the other hand, lowest response was reported on treatment Tithonia 50 kg N ha^{-1}, control and Tithonia 50 kg N ha^{-1} + Urea 50 kg N ha^{-1} for aluminum, phosphorus and calcium, respectively. The treatments significantly influenced the percentage of polyphenols, vitamin C and the antioxidant capacity of Swiss chard while no significance was reported for total soluble solutes. The polyphenols and Vitamin C content were highest on Tithonia 100 kg N ha^{-1} and control respectively. However, the control and Tithonia 100 kg N ha^{-1} were not significantly different for both phytochemical. Low polyphenol and Vitamin C contents were recorded on VC 50 kg N ha^{-1} and VC 100 kg N ha^{-1}, respectively. A similar trend was observed for antioxidant activity since a positive correlation was observed between the polyphenols and vitamin C and the antioxidant activity. The results demonstrate that there is potential for the use of Tithonia as an organic source of N to grow good quality Swiss chard rich in health promoting phytochemicals.

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