DIFFERENTIAL IMPACT OF NITROGEN FORMS ON SELECTED PHYTOCHEMICALS AND OXALATE CONTENTS IN THREE VEGETABLE AMARANTH VARIETIES IN KENYA

Rozy Munene[1]*, Evans Changamu[2], Nicholas Korir[1], Joseph P Gweyi-Onyango[1]
[1]Department of Agricultural Science and Technology, Kenyatta University, Nairobi, Kenya
[2]Department of Chemistry, Kenyatta University, Nairobi, Kenya.
Kenyatta University, Nairobi, Kenya
*E-mail: rozymunene@gmail.com

Abstract
Amaranthus are a great source of phytochemicals important for nutritive and remedial benefits. However they *are also great accumulators of anti-nutrients such as oxalates. Nitrogen (N), a vital plant nutrient is a strong determinant of plant nutritive value. The study therefore investigated the total flavonoids content (TFC) and phenolic content (TPC) accumulation, antioxidant activity and oxalates content in relation to different N forms in three vegetable amaranth varieties. Three N forms were used; ammonium, nitrate, ammonium/nitrate mixture {ammonium and ammonium nitrate were stabilized with Piadin® as nitrification inhibitor} and control on three amaranth varieties were AB5, AB6 and AB7 in randomized complete block design replicated three times. Sole ammonium and the control enhanced accumulation of both TFC and TPC, compared to the nitrate and ammonium nitrate mixture. Under ammonium treatment, TFC increased by 13.8% in AB5, 17.4% in AB6 and 14.7% in AB7 while TPC increased by about 19.5% in AB5, 23% in AB6 and 20% in AB7 in greenhouse. Similar trends were observed from the field experiment. Likewise, NH_4^+ - N form had higher antioxidant DPPH scavenging activity indicated by high inhibiting capacity and lower IC_{50} value (concentration which scavenged 50% of the DPPH radicals). Compared to control, nitrate elevated oxalate accumulation unlike ammonium treatment which on contrary inhibited oxalate buildup. It was therefore concluded that TFC, TPC and antioxidant capacity increased with N deficiency and ammonium-N provision while oxalate content was enhanced under nitrate treatment in leafy amaranth. This is relevant for nutritive wellbeing in human beings.*

Key words: Amaranth, Nitrate, Ammonium, Flavonoids, Phenolics Oxalate and varieties

Submitted: 20.03.2017 Reviewed: 22.05.2017 Accepted: 02.06.2017

1. INTRODUCTION

Amaranth (*Amaranthus spp*) is one of the widely cultivated and commonly consumed African leafy vegetables (ALV) in African households (Munene et al., 2016). The species has a rapid growth habit and can be harvested within a short time (3 to 4 weeks) after sowing and possesses short harvesting interval (Onyango et al., 2012). This makes the leafy vegetable appropriate to resource-poor farmers as it offers cheap means of income generation and employment opportunities along the production chain, thus economic security, especially in the peri-urban proximities (Munene et al., 2016). In addition, biochemical analysis of amaranth has revealed that the plant contain considerable levels of essential vitamins, mineral elements and dietary fibre. Moreover, amaranths are excellent hosts of biologically active polyphenolic compounds such as phenolics and flavonoids associated with health and therapeutic benefits (Amabye 2015; Kwenin et al. 2011). These remedial properties are strongly linked to the superior anti-oxidative capacity of the phytochemicals to a certain extent than from the vitamin C and Beta-catotene (Khandaker et al., 2008). Maisarah et al. (2013); reported a positive relationship between secondary metabolites and antioxidant activity in some vegetables such as amaranth and fruits. Research on phytochemicals has currently risen due to the fact that consumption of these phytochemicals together with vitamins and minerals has been shown to reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related problems (Amabye 2015). This presents leafy amaranth as an important vegetable towards attaining

nutrition, economic and food security (Munene et al., 2016). However, the nutritive value of *Amaranthus* may be compromised as they accumulate high levels of anti-nutrients such as oxalates (Onyango et al., 2012). Evidence indicates that oxalates play diverse functional roles in plants, including calcium regulation, plant protection and detoxification of certain heavy metals (Weir et al., 2006). Despite the physiological benefits in plants, large amounts of oxalates in any edible parts of vegetables and food crops not only lower their nutritional values but also pose health risks to humans. Upon ingestion, oxalates may cause adverse effects like corrosion of the gastrointestinal tract and gastric hemorrhage. Precipitation of oxalates with calcium is likely to restrict the nutrients availability which may be of concern especially for women and children, who require greater levels of calcium in their diets. Oxalates also increase the probability of developing kidney stones (Nakata, 2003).

Nitrogen (N) is an indispensable mineral element and its availability is a strong determinant of plant growth and development (Qiang et al., 2014). It influences both the primary and secondary metabolic pathways thus secondary plant metabolites accumulation (Chen et al. 2011). Nitrogen is taken up by the plants in two major forms; ammonium (NH_4^+) cation and nitrate (NO_3^-) anion (Sabir et al., 2013) and these forms influence biomass production as well as nutritional quality such as oxalate accumulation (Liu et al., 2015; Rahman and Kawamura 2011). For this reason, the effects of nitrogen forms on oxalate levels in leafy amaranth should be paid attention to. Higher plants enhance phytochemicals build-up believed to act as defense compounds against various environmental constraints such as nutritional related stresses (Nakabayashi and Saito, 2015). Deficiency of vital elements like nitrogen has been found to enhance accumulation of phenolic compounds in the plant tissues (Ibrahim et al. 2011). While some work have been reported on impact of different N levels phytochemical accumulation (Argyropoulou et al. 2015; and Salahas et al. 2011), literature on effects of different N forms on phytochemical accumulation in plant tissues is scanty. The present study was therefore to evaluate the levels of accumulation of total flavonoids and phenolic compounds and antioxidant activity in leafy amaranth grown in different N forms. Effects of the N sources on oxalate content were also investigated.

2 MATERIALS AND METHODS

2.1 Experimental Design and Treatments

The experiments were laid out in a split plot arrangement on a Randomized Complete Block Design (RBCD) replicated three times. The main plot comprised of three vegetable amaranth varieties (AB5, AB6 and AB6) while three N-forms; sole NO_3^-, sole $NH_4^+$and NH_4/NO_3 mixture and control (no N added) comprised the sub-plots. Sole NH_4^+ and NH_4/NO_3 were stabilized with Piadin® as the nitrification inhibitor composed of a mixture of dicyandiamide and 3, 4 methylpyrazole phosphate.

2.2 Planting Material

Three amaranth seeds were sourced from Jomo Kenyatta University of Agriculture and Technology (JKUAT) and planted at the Agricultural Science and Technology (AST) Research and Demonstration Farm Kenyatta University, Kenya. The seeds were directly sown at a spacing of 10cm by 30cm in a plot measuring 3 by 4 m, separated by 1m path in the field while 2kg containers filled with media were used for the greenhouse experiment. Triple superphosphate (TSP) was used as basal fertilizer.

2.3 Harvesting, Data Collection, and Preparation of Plant Samples

Harvesting of the plant samples was done by uprooting the whole plant where stratified sampling was used four weeks after treatment. The shoots were oven dried at 60-65^{0}C for 72 hours until the weight was constant. The dried plant samples were ground using a grinder-MIKA® to fine powder (0.2mm) and kept in zip lock polythene bags, appropriately labeled and stored until phytochemical and anti-nutrient(oxalate) analysis.

2.4 Extraction of plant material

Methanolic extraction was used on the oven-dried plant samples. About 20g of the powdered plant material was placed in a flask, covered with 100ml methanol AR and allowed to stand for about 48–72 hours. Filtering was done using Whatman filter paper No. 1 and distilled to obtain methanol-free paste using rotary evaporator at 65°C (Mibei et al. 2012). The resulting extracts were properly labeled and preserved at 5°C in airtight plastic vials for analysis.

2.5 Total flavonoid contents analysis

Aluminium trichloride ($AlCl_3$) method was used for the determination of TFC of the sample extracts (Mervat et al. 2009). Portions of 1.5 ml of 1:10g.ml^{-1} extracts were added to equal volumes of a solution of 2% AlCl3 $6H_2O$. The mixture was vigorously shaken and allowed to stand for about 10–15 minutes and absorbance recorded using spectrophotometer (Spectro SC labmed inc.) at 425 nm. Flavonoid contents were expressed as mg catchin equivalent (mgCE /g) dry weight.

2.6 Total phenolic contents Analysis

Folin Ciocalteu reagent was used to determine total phenolic content (TPC) in plant sample (Esmaeli et al. 2009, Nabavi et al. 2008). Dilute solution of amaranth extracts (0.5 ml of 1:10 g.ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous 5% Na_2CO_3 (4 ml). The mixture was allowed to stand for 15 minutes and absorbance read at 765 nm with a spectrophotometer (Spectro SC labmed Inc.). Gallic acid (GA) was used to obtain the total phenolic concentration by preparing a standard curve at 0, 50, 100, 150, 200, and 250 mg ml^{-1} concentrations GA. Total phenolic content values were expressed in terms of gallic acid equivalent (mgGAE/g) of dry weight.

2.7 Anti-oxidant analysis

Radical-scavenging capacity of samples was used to determine using Diphenylpicryl hydrazyl (DPPH) according to (Mibei et al. 2012). Different concentrations 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg.ml^{-1} of the extracts were prepared using methanol. One ml of the extract was placed in a test tube, 3ml of methanol added followed by 0.5ml of 1 mM DPPH in methanol. This was shaken vigorously and left to stand for about five minutes. Concentrations of ascorbic acid were prepared at the same concentrations as the extract and used as a standard. Blank solution was prepared with same amount of DPPH and methanol. The absorbance of the solutions was obtained at 517 nm with a spectrophotometer (Spectro SC labmed Inc.). Radical scavenging capacity of the samples was determined using the formula:

% inhibition = {[Ab-Aa]/Ab} x 100

Where: Ab = absorption of the blank sample, Aa = absorption of the extract

2.8 Oxalate Analysis

Determination of oxalates was done by HPLC (Yu et al, 2002). Aliquats of 0.5g, sample was homogenized in 4 ml of 0.5 N HCl. The mixture was heated at 100 °C for 30min with intermittent shaking. To the homogenate distilled water was added up to a volume of 25 ml. About 3 ml of the solution was obtained and centrifuged at 12,000 rpm for 15 min. About 1 ml of supernatant was passed through a filter (0.45 µm) before HPLC analysis. Standards were prepared at varying concentrations for quantification. Hypsil C18 column (5 µM, 4.6 mm x 250 mm) equipped Waters 550 was used as the static phase and the mobile phase was a solution containing 0.5% KH2PO4 and 0.5 mM TBA (tetrabutylammonium hydrogen sulphate) buffered at pH 2.0 with orthophosphoric acid. Flow rate was 1 ml min^{-1} and detection wavelength was at 220 nm.

2.8 Data analysis

Data was subjected to analysis of variance (ANOVA) at 95% confidence level using SAS-computer software (SAS 2015; Version 9.0).Where significant, further mean separation was done by LSD.

3. RESULTS AND DISCUSSION

3.1 Total flavonoids and phenolics concentration

Total shoot flavonoids contents were significantly ($P \leq 0.05$) influenced by different

nitrogen forms, in both greenhouse and field experiments. The results revealed a stimulatory effect of sole ammonium on TFC content in amaranth plants (Table 1). Compared to ammonium, nitrate reduced TFC by 13.8% in AB5, 17.4% in AB6 and 14.7% in AB7 in greenhouse and 16.6% in AB5, 19.6% in AB6 and 17.9% in AB7 in the field experiment. In relation to control, ammonium reduced TFC by 9.8% in AB5, 10.6% in AB6 and 12.7% in AB7 in the greenhouse and 8.4% n AB5, 10.8% in AB6 and 7% in AB7 in the field experiment while nitrate had a more reduction of TFC by 22.3% in AB5, 26.2% in AB6 and 25.5% in AB7 in greenhouse and 23.6% in AB5, 27.7% in AB6 and 22.9% in AB7 in the field experiment. The results indicated a notable interaction between N forms and amaranth varieties for TFC in the greenhouse experiment (Table 1) where AB7 variety showed maximum (30.6mgg^{-1} under control, 26.7mgg^{-1}under sole NH_4^{+}) concentration while AB5 (26.5mgg^{-1} under control and 23.9mgg^{-1} under sole NH_4^{+}) had minimum shoot content. Just like TFC, ammonium -treated plants significantly ($P \leq 0.05$) increased phenolic content compared to those supplied with nitrate (Table 1). Under ammonium treatment, TPC increased by about 19.5% in AB5, 23% in AB6 and 20% in AB7 in greenhouse 14% in AB5 and AB7 and 16% in AB6 in the field experiment compared to the nitrate as only treatment in amaranth plants. Amaranth plants not treated with any N (control) had comparatively higher TFC (25.5mgg^{-1} for AB5, 28.2mgg^{-1} for AB6 and 30.6mgg^{-1} for AB7) to ammonium form (23.9mgg^{-1} for AB5, 25.2mgg^{-1} for AB6 and 26.7mgg^{-1}) in the greenhouse while accumulation of TPC in the plants treated with ammonium form were not statistically different with the control both in the greenhouse and field experiment except for AB7 (79.9mgg^{-1} under Am and 74.7mgg^{-1}) in greenhouse.

Table 1: Effects of N forms on TFC and TPC accumulation in three amaranth varieties

Treatments		Total Flavonoids content (mg/g CE)		Total Phenolics content (mg/g GAE)	
		Grnhse	Field	Grnhse	Field
AB5	**Cntl**	26.5^{c}	17.8^{bc}	69.8^{bc}	51.5^{bcd}
	Am	23.9^{e}	16.3^{cde}	67.6^{c}	50.5^{cd}
	AmNi	21.5^{f}	14.1^{gh}	58.2^{e}	40.7^{f}
	Ni	20.6^{f}	13.6^{h}	54.4^{f}	43.4^{ef}
AB6	**Cntl**	28.2^{b}	19.5^{a}	72.6^{b}	51.8^{b}
	Am	25.2^{d}	17.6^{cd}	75.5^{b}	57.4^{ab}
	AmNi	23.1e	15.6^{efg}	61.4^{d}	46.7^{def}
	Ni	20.8^{f}	14.1^{fgh}	58.1^{e}	48.2^{d}
AB7	**Cntl**	30.6^{a}	19.2^{ab}	74.7^{b}	61.4^{a}
	Am	26.7^{c}	17.8^{bc}	79.9^{a}	63.3^{a}
	AmNi	23.0^{e}	16.1^{def}	64.2^{d}	52.3^{cd}
	Ni	22.8^{e}	14.8^{efgh}	63.9^{d}	54.6^{bc}
P value		0.001	0.003	0.001	0.003
LSD		1.1	1.5	3.0	6.2
N x V		*	NS	NS	NS

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate * Significant values at ($P \leq 0.05$) and NS- Not significant. N x V (interaction between Nitrogen and Variety).

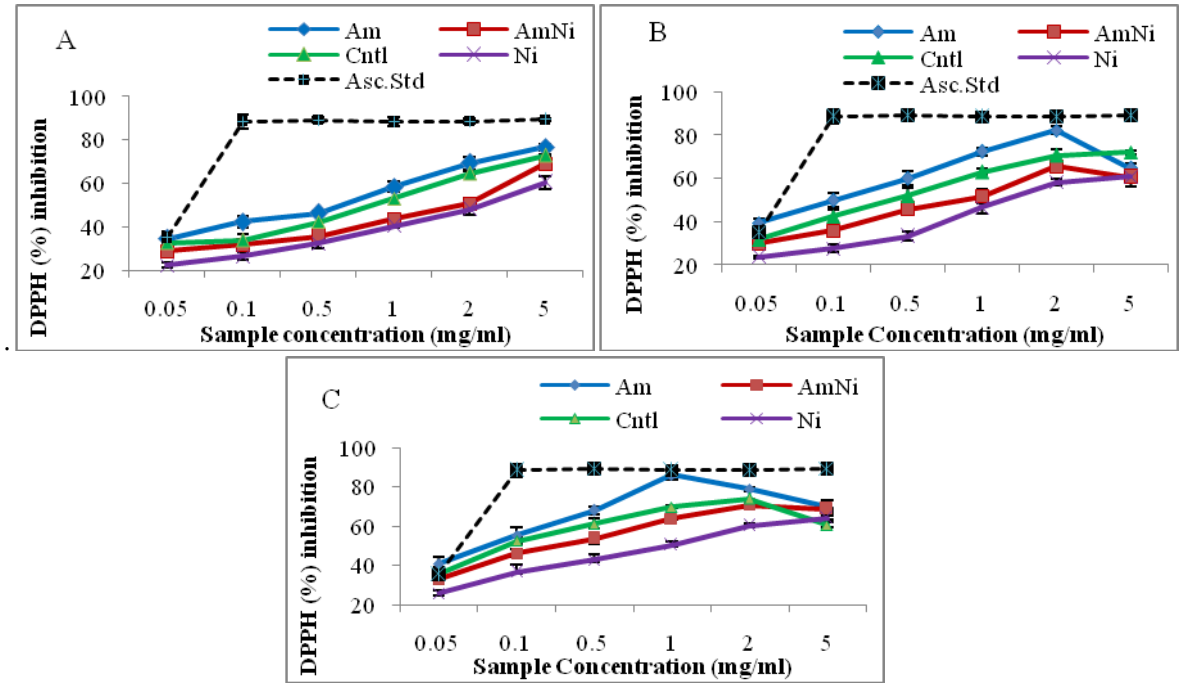


Fig. 1: Effects of Nitrogen forms of three amaranth varieties (A represents AB5, B represents AB6 and C represents AB7) on anti-oxidant DPPH scavenging activity in greenhouse experiment

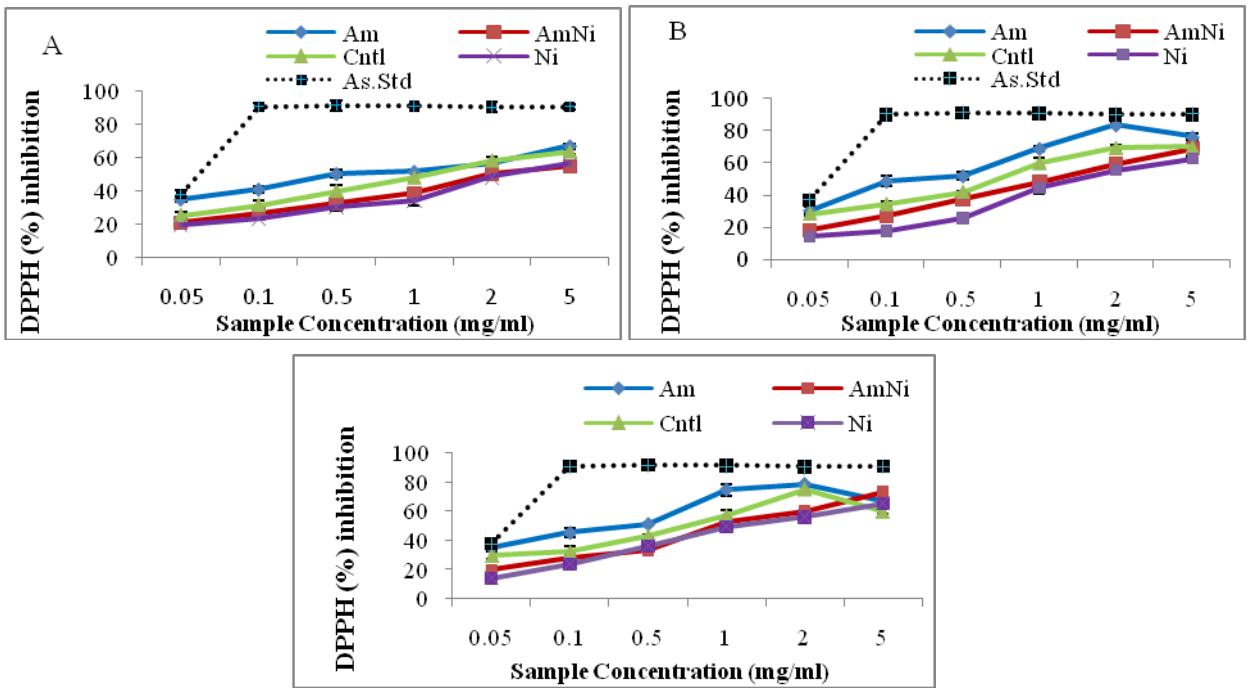


Fig. 2: Effects of Nitrogen forms of three amaranth varieties (A represents AB5, B represents AB6 and C represents AB7) on anti-oxidant DPPH scavenging activity in field experiment

Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

Table 2: IC_{50} and maximum percentage inhibition values for the three amaranth extracts

Treatments		Greenhouse Experiment			Field Experiment		
		IC_{50} ($mg/g_{)}$	Max In. (%)	Conc (mg/g)	IC_{50} ($mg/g_{)}$	Max In. (%)	Conc (mg/g)
AB5	Cntl	0.8	72.0	5	1.0	63.7	5
	Am	0.7	69.4	2	0.5	67.0	5
	AmNi	2.0	69.5	5	2.0	54.7	5
	Ni	2.5	59.4	5	2.1	56.3	5
AB6	Cntl	0.4	82.0	2	0.7	70.3	5
	Am	0.3	67.8	2	0.2	76.7	2
	AmNi	1.0	60.9	5	1.0	70.3	5
	Ni	1.8	68.0	2	1.1	62.9	5
AB7	Cntl	0.07	72.0	2	0.6	74.7	2
	Am	0.06	86.1	1	0.5	78.4	2
	AmNi	0.5	70.1	2	0.9	73.4	5
	Ni	0.7	64.3	5	1.0	65.3	5

Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

Variety AB7 was the superior in TFC and TPCs, followed by AB6 then AB5 both in the greenhouse and field experiment under all the nitrogen treatments. Higher concentrations of TFC and TPC were observed in the greenhouse compared to the field experiment. The current study indicated that when no N (control) was supplied; this up-regulated flavonoid and phenolic levels in the amaranth plants. This agrees with the findings of Ibrahim et al, (2011) which show that the accumulation of polyphenolic components in plant tissues is often enhanced under conditions of nitrogen nutrition deficiency. Application of plants with sole ammonium source leads to acidification of rhizosphere associated with poor plant growth (data not shown). This probably might have induced plant defense mechanisms by elevated polyphenolic accumulation as defense strategy against nutritional stress. In addition the authors speculate the large amount of phytochemicals could be as a result of increased occurrence of polyamines (Chen *et al.,* 2011) which act as precursors of shikimic acid pathway associated with secondary metabolites biosynthesis.

3.2 Anti-oxidant DPPH scavenging activity

Nitrogen forms had a significant ($P \leq 0.05$) influence on the anti-oxidant DPPH inhibition activity (Figures 2 and 3). The scavenging activity of all the extracts on DPPH radicals increased as the concentration increased in the range of 0.05 – 5.0 mg/ml

Ammonium as the only N source exhibited superior antioxidant DPPH scavenging activity indicated by lower IC_{50} value (concentration which scavenged 50% of the DPPH radicals) (Table 2). Under ammonium treatment, variety AB7 had the most effective DPPH inhibiting activity with lowest IC_{50} (0.06mg/g), followed by AB6 with IC_{50} of 0.3mg/g while AB5 had the highest IC_{50} of 0.7mg/g. While the samples from plants supplied with no N (control) had almost equivalent DPPH inhibiting capacity to the sole ammonium; the sole nitrate and ammonium/nitrate mixture treatment on the other hand had lower scavenging capacities indicated by high IC_{50} value. Specifically, nitrate as sole treatment had a relatively poor inhibiting capacity of DPPH radicals with higher IC_{50} values of about 0.7mg/g (AB7), AB6 1.8mg/g for AB6 and AB5 had 2.5mg/g compared to ammonium/nitrate with IC_{50} values of 0.5mg/g for AB7, 1.0mg/g for AB6 and 2.0 mg/g for AB5 in the greenhouse experiment. Similar trend was observed in the field experiment where AB5 and AB7 had a IC_{50} of 0.5mg/g, whereas AB6 (0.2mg/g) had lower IC_{50} under exclusive ammonium treatment, however DPPH inhibiting capacity was superior for the greenhouse amaranth

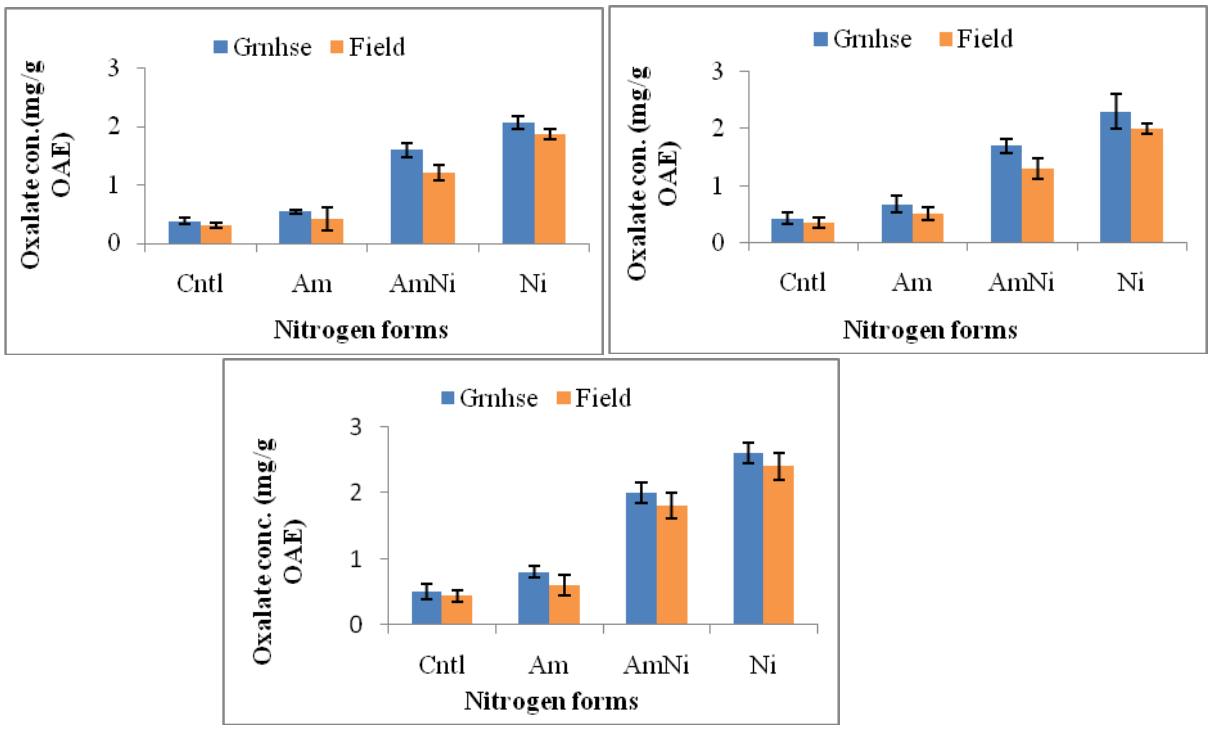
sample extracts compared to the field experiment sample extracts. Regarding percentage inhibition capacity, variety AB7 was still superior as it showed maximum inhibition percentage of (86.1%) in concentration of 1.0 mg/g and 78.4% in 2mg/g in greenhouse and field sample extracts respectively.

Similar to the TFC and TPC accumulation, plants supplied with NH_4^+-N exhibited superior scavenging capacity unlike other (NO_3^- and NH_4^+/NO_3^- mixture). Plants exposed to high NH_4^+ concentration as N source accumulate low molecular osmolytes among them polyamines (Clausen et al,. 2006) which enhances the plants tolerance to stresses (Tassoni et al., 2008) thus possibly the observed superior antioxidant activity. Phytochemicals such as flavonoids and phenolics constitute a major group of compounds that act as primary antioxidants. The current results agree with findings Ogembo, (2015) which presents them as effective antioxidants or free radical scavengers in leafy vegetables.

3.3 Oxalate Accumulation

Nitrogen forms significantly (P≤ 0.05) affected oxalate concentration in the greenhouse and field experiments (Figure 3). Amaranth plants supplied with nitrate-N considerably increased accumulation of oxalate compared those fed on ammonium N source. While amaranth plants not provided with any N (control), had minimum accumulation of oxalates, those treated with nitrate as the sole N source markedly stimulated oxalate concentration by above 80% in all the varieties; both in the greenhouse and field experiments while ammonium as the sole treatment increased oxalate content by 29.6% in AB5, 37.3% in AB6 and 37.5% in AB7 varieties in the greenhouse.

Similar trend was observed in the field experiment. Present results showed that reduction in nitrate N form restricted oxalate accumulation in the amaranth plants. This was evident by provision of ammonium/nitrate mixture which indicated lower oxalate content as compared to sole nitrate in all the varieties both in the greenhouse and field experiment.



Cntl- control, Am – Ammonium, AmNi - Ammonium nitrate, Ni- nitrate

Fig. 3: Effects of Nitrogen forms on Oxalate accumulation in the three amaranth varieties (A repesented-AB5, B prepresented-AB6 and C repesented- AB7) in greenhouse and field experiment

Variety AB7 had the highest level (2.6 mg/g OAE and 2.4mg/g OAE) of oxalate while AB5 (2.1mg/g OAE and 1.9mg/g OAE) accumulated the least levels of oxalates under sole nitrate treatment in the greenhouse and field experiment accordingly. This is in line with the findings of Liu et al., (2014) who reported that increased oxalate accumulation is linked with supply of nitrate and not ammonium – N source.

4. CONCLUSION

Amaranth plants responded differently to different N forms. Ammonium as sole N treatment increased total flavonoids and phenolics accumulation as well as anti-oxidant inhibiting capacity of amaranth extracts unlike nitrate and ammonium/nitrate treatments while on contrary oxalate accumulation was enhanced by nitrate treatment in vegetable amaranth. The ammonium form showed to be superior in terms of improving amaranth quality and hence improving health benefits.

5. ACKNOWLEDGEMENTS

We sincerely thank Kenyatta University- Vice Chancellor's research Grant Programme for funding the study. We also wish to genuinely thank Prof Christof Engels of Humboldt University, Berlin for providing Padin® and Prof Abukutsa-Onyango of JKUAT for the provision of amaranth seeds.

6. REFERENCES

[1] Amabye TG. Evaluation of Physiochemical, Phytochemical, Antioxidant and Antimicrobial Screening Parameters of *Amaranthus spinosus* leaves. Natural Products Chemistry and Research 2015; **4** (199): 1-5

[2] Argyropoulou K, Salahas G, Hela D and Papasavvas A. Impact of nitrogen deficiency on biomass production, morphological and biochemical characteristics of sweet basil (*Ocimum basilicum* L.) plants, cultivated aeroponically. Journal of International Scientific Publications. Agrciculture and food 2015; **3**: 32–42

[3] Chen L, Liu QQ, Gai JY, Zhu YL, Yang LF and Wang C. Effects of nitrogen forms on the growth and polyamine contents in developing seeds of vegetable soybean. *Journal* of Plant Nutrition 2011; **2**(34):504–521.

[4] Claussen W, Brückner B, Krumbein A and Lenz F. Long-term response of tomato plants to changing nutrient concentration in the root environment—the role of proline as an indicator of sensory fruit quality. Plant Science. 2006; **171**:323–331.

[5] Esmaeili A, Tavassoli A, and Ebrahimzadeh MA. Antioxidant activity and free radical scavenging activity of S*alvia glutiosa* growing in Iran. Pharmacology Online. *2009;* **2**: 109–116

[6] Ibrahim MH, Jaafar HZ, Rahmat A and Rahman ZA (2011) Effects of Nitrogen Fertilization on Synthesis of Primary and Secondary Metabolites in Three Varieties of Kacip Fatimah *(Labisia Pumila* Blume). International Journal of Molecular Sciences **12**:5238–5254

[7] Khandaker L, Ali MB, and Oba S. Total polyphenol and antioxidant activity of red amaranth (*Amaranthus tricolor* L.) as affected by different sunlight level. Journal of the Japanese Society for Horticultural Science. 2008; **77(**4):395-401.

[8] Kwenin WK, Wolli M and Dzomeku B.M Assessing the nutritional value of some African indigenous green Leafy Vegetables in Ghana. Journal of Animal and Plant Sciences 2011; **10**(2): 1300–1305.

[9] Liu X, Lu L, Chen Q, Ding W, Dai P, Hu Y, Yu Y, Jin C. and Lin X. Ammonium reduces oxalate accumulation in different spinach (*Spinacia oleracea* L.) genotypes by inhibiting root uptake of nitrate. Food chemistry. 2015; **186**:312-318.

[10] Maisarah AM, Nurul Amira B., Asmah R. and Fauziah O. Antioxidant analysis of different parts of Carica papaya. International Food Research Journal 2013; **20**(3): 1043-1048

[11] Mervat M, El Far M and Taie HA. Antioxidant Activities, Total Anthocyanins, Phenolics and Flavonoids Contents of Some Sweet potato Genotypes under Stress of Different Concentrations of Sucrose and Sorbitol. Australian Journal of Basic and Applied Sciences. 2009; **3**(4): 3609–3616.

[12] Mibei EK, Ojijo, NK, Karanja SM and Kinyua JK. Phytochemical and antioxidant analysis of methanolic extracts of four African indigenous leafy vegetables. Annals Food Science and Technology. 2012; **13**: 37–42.

[13] Munene R, Changamu E, Korir N. and Gweyi-Onyango JP. Characterization and Documentation of Factors Contributing to Production and Consumption of African Leafy Vegetables (ALVs) in Kiambu and Kirinyaga Counties in Kenya. Asian Research Journal of Agriculture 2016; **1** (3): 1-9.

[14] Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia A. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica* Mey. Pharmacologyonline. 2008; **2**: 560–567

[15] Nakabayashi R and Saito K. Integrated metabolomics for abiotic stress responses in plants. Current Opinion in Plant Biology 2015; **24**: 10–16.
[16] Nakata PA. Advances in our understanding of calcium oxalate crystal formation and function in plants. Plant Science. 2003; **164**: 901–909.
[17] Ogembo JO. Effects of Phosphorus Deficiency on Secondary Metabolites and Distribution of African Nightshade in Siaya and Kisii Counties, Kenya. Master's thesis. 2015. Kenyatta University, Nairobi, Kenya.
[18] Onyango CM. Harbinson J, Imungi JK, Onwonga RN, and van Kooten O. Effect of nitrogen source, crop maturity stage and storage conditions on phenolics and oxalate contents in vegetable amaranth (*Amaranthus hypochondriacus*). Journal of Agricultural Science, 2012; **4**(7), 219- 230
[19] Qiang ZH, Fei XU, Rong-fu WA, Liang-zuo SH, Rui LI. and De-yu ZH. Effects of nitrogen forms on the growth, yield and fruit quality of tomato under controlled alternate partial root zone irrigation. Yingyong Shengtai Xuebao. 2014; **25**(12): 3547-3555
[20] Rahman, MM. and Kawamura O. (2011). Oxalate Accumulation in Forage Plants: Some Agronomic, Climatic and Genetic Aspects. Asian-Aust. Journal of Animal Sci. **24**(3): 439 – 448
[21] Sabir M, Hanafi MM, Malik MT, Aziz T, Zia-ur-Rehman M, Ahmad HR, Hakeem KR and Shahid M. Differential effect of nitrogen forms on physiological parameters and micronutrient concentration in maize (*Zea mays* L.). Australian Journal of Crop Science 2013; **7**(12):1836–1842
[22] Salahas G, Papasavvas A, Giannakopoulos E, Tselios T and Savvas D. Impact of nitrogen deficiency on biomass production, gas exchange, and betacyanin and total phenol concentrations in red beet (*Beta vulgaris* L.) plants. European Journal of Horticultural Science. 2011; **76**(5): 194–200.
[23] Weir TL, Bais HP, Stull VJ, Callaway RM, Thelen GC, Ridenour WM, Bhamidi S, Stermitz FR and Vivanco JM. Oxalate contributes to the resistance of *Gaillardia grandiflora* and *Lupinus sericeus* to a phytotoxin produced by *Centaurea maculosa*. Planta; 2006; **223**(4):785.
[24] Yu L., Peng XX, Yang C, Liu YH. and Fan YP. Determination of oxalic acid in plant tissue and root exudates by reversed phase high performance liquid chromatography. Chinese Journal of Analytical Chemistry. (2002); **30**: 1119–1122.