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Dharmendranaik E
Ph.D. Scholar, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellanikkara, Thrissur, Kerala Agricultural University, Kerala, India

Durga Devi KM
Professor (Rtd.), Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellanikkara, Thrissur, Kerala Agricultural University, Kerala, India

Beena VI
Assistant Professor and Head, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellanikkara, Thissur, Kerala Agricultural University, Kerala, India

Geetha P
Assistant Professor, Department, of Soil Science and Agricultural Chemistry, (Rediotracer Laboratory), College of Agriculture, Vellanikkara, Thrissur, Kerala Agricultural University, Kerala, India

Corresponding Author:
Dharmendranaik E
Ph.D. Scholar, Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellanikkara, Thrissur, Kerala Agricultural University, Kerala, India

Effect of nutrient management practices on nitrate and oxalate content in cooked and raw amaranth (*Amaranthus tricolor* L.)

Dharmendranaik E, Durga Devi KM, Beena VI and Geetha P

Abstract
An experiment was conducted at Kerala Agricultural University, College of Agriculture, Vellanikkara in 2019 to investigate the effects of nutrient management practices on the levels of nitrate and oxalate in cooked and raw amaranth. The study employed a factorial completely randomized design with five nutrient management treatments and two types of soils (black soil and lateritic soil). The results indicated significant variation in the levels of nitrate and oxalate, which are anti-nutrients, in both cooked and raw amaranth. Cooking was found to reduce the content of nitrate (33.83 to 2.27%) and oxalate (20.13 to 11.20%). The nitrate and oxalate contents in cooked amaranth ranged from 498.66 to 2126.66 mg/kg and 2130.94 to 3361.49 mg/kg, respectively, while in raw amaranth, the nitrate and oxalate contents ranged from 1056.00 to 2229.33 mg/kg and 2641.17 to 4055.12 mg/kg, respectively. Among the soil types, lateritic soil (Alfisol) registered significantly lower levels of nitrate and oxalates in cooked as well as raw amaranth. Among the treatments, T_1 (organic KAU-POP+FYM 25t/ha) had recorded significantly lower amounts of nitrate. In the case of raw samples it was on par with T_5- (soil test-based application of nutrients with FYM 25 t/ha). In the case of oxalate, cooked samples of T_1 registered significantly lower value which was also on par with T_5. Oxalate content in raw samples of T_1 was also significantly lower than the other treatments. The data obtained from the experiment also indicated significant variations in amaranth yield across different soil types and nutrient management practices as well as their interaction. Among the treatments, the one receiving T_4 (NPK-100:50:50 in the form of Factamphos and MOP, along with FYM-25t/ha) showed a significantly higher yield in both soil types. The yield from this treatment was similar to that of T_2 (KAU-POP NPK-100:50:50 with urea, SSP, MOP + FYM- 25t/ha) and T_3 (NPK-100:75:75 with urea, SSP, MOP + FYM-25t/ha + lime based on soil test). The lowest yield was observed in T_1 (organic KAU-POP) and soil test-based application of nutrients with FYM-25t/ha (T_5) exhibited comparable yield to that of T_2 and T_3. The results indicated clearly that the application of organic manure alone is a good practice to reduce the nitrate and oxalate content in amaranth. However, yield is a limiting factor for organic POP treatment. Among the integrated nutrient management practices, soil test-based application of nutrients + FYM-25 t/ha showed superiority over the other treatments in reducing the contents of anti-nutrients in amaranth along with reasonably good yield.

Keywords: Anti-nutrients, nitrate, oxalate, cooked and raw amaranth, lateritic soil, black soil, KAU-POP, FYM, urea, Factamphos, SSP, and MOP

Introduction
Amaranth (*Amaranthus spp*.) is one of the most important leafy vegetable in the tropical region of the world. It is commonly known as "cheera" in Malayalam and cultivated in different parts of South India *viz*., Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, and Maharashtra under varying soil and climatic conditions. Amaranth belongs to the family Amaranthaceae which is comprised of 65 genera and 850 species. *Amaranthus tricolor* is a native of South and South-East Asia. Rai and Yadav (2005) [7] reported that tender stems and leaves contains moisture (85.70%), protein (4%), carbohydrate (6.3%), fat (0.5%), vitamin C (99 mg/100 g), carotenoids (90-200 mg/kg) and minerals like calcium (397 mg), iron (25.5mg), zinc, magnesium and phosphorus (83.0 mg) and trace elements and it is also a good source of dietary fiber. Amaranth has bioactive components such as antioxidants, antimalarial and antiviral properties, which prevent cancer, cardiovascular diseases, diabetes, etc., and also help protect the body from long-term degenerated diseases (Raheena, 2007) [6]. The short duration nature, quick response to manures and fertilizers, high yields and adaptability to various agro climatic conditions makes it commercially important leafy vegetable crop. However presence of anti-nutrients like nitrates and oxalates hinder the large-scale production and consumption of amaranth. Nitrate and oxalate make many health problems like

interference with normal functioning of haemoglobin (Methemoglobinemia or blue-baby syndrome under higher levels of nitrate) and kidney stone formation due to high oxalate etc. Permissible limit of nitrate in vegetables is 2220 mg/kg. Acceptable Daily Intake (ADI) for nitrate is less than 3.7 mg/kg of body weight (equivalent to 222 mg nitrate per day for a 60 kg adult) (EFSA, 2008) [2]. Permissible limit for oxalate is 2500 mg/kg fresh sample (Oguchi, *et al.,* 1996) [5].

Nitrogen fertilizer is known as one of the plant nutrients that influence vegetative growth in most crops and subsequently increased yield. Nitrate content of vegetables grown under integrated nutrient practice was lower than pure chemical fertilizer. The nitrate and oxalate contents in plants are influenced by plant metabolism, age of the plant, environmental factors, the amount of available nitrate in the soil etc. (Anjana *et al.,* 2007) [1].

The leafy vegetables are generally consumed in cooked form in India. Amaranth is also consumed after cooking. A large number of studies have been conducted in various parts of the world to determine the effects of cooking and processing on the nitrate and oxalate contents. High nitrate and oxalate-containing vegetables should always be cooked to reduce the nitrate and oxalate content; soaking vegetables prior to cooking will also reduce the oxalate contents by leaching (Noonan, *et al*., 1999) [4].

The data published in the state of Kerala is meagre on the effect of cooking of leafy vegetables on their anti-nutrient contents. Therefore, the present study is focused on the effect of different fertility management practices on nitrate and oxalate content in cooked and raw amaranth and also the effect of these treatments on amaranth yield in the major amaranth growing areas of Kerala state *viz*., Chittur black soil (Palakkad district) and lateritic soil (Thrissur district).

Materials and Methods

A pot culture experiment was conducted at College of Agriculture, Vellanikkara during the period from January to May (2019). Two types of soils were selected for study, one is black soil from Chittur area coming under the soil order Vertisol (USDA- Typic Haplusterts) and the second is lateritic soil coming under the soil order Alfisol (USDA-Kandic Paleustalfs). Composite samples of soils from 0-15 cm depth were collected from randomly selected points, bulked, air dried and sieved through 2 mm sieve. Basic physico-chemical characteristics of the processed samples were analysed for pH, EC, OC, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B for calculating the amount of nutrients to be applied in each treatment. Mud pots were filled with five-kilogram soil and required amount of FYM, lime and fertilizes were applied as per treatments (Table 1). Red amaranth variety "Aruṅ" was selected for study and the seeds were purchased from Department of Vegetable Science, College of Agriculture, Vellanikkara, Kerala Agricultural University. Amaranth seeds were sown in the tray containing the mixture *viz*., soil: vermicompost: cow dung in the ratio of 1:1:1. Adequate amount of water was given at regular intervals (KAU, 2016). Twenty-four days old seedlings were transplanted to the pots with appropriate treatments. Pest and diseases were noticed during crop growth stage and appropriate control measures were taken. Harvesting was done at 31 days after transplanting and yield was recorded. The harvested plant samples were washed and used for analysis of anti-nutrients like nitrate and oxalate before and after cooking. Edible plant parts were cut into small pieces of 1-2cm and air dried for estimating anti-nutrients in the raw amaranth. Similarly small quantity of the harvested plant parts were steamed for four minutes, air dried and the anti-nutrient analysis was carried out as per standard procedures.

Table 1: Chemical properties of soil used for pot culture experiment

Parameter	Black soil	Lateritic soil
pH	8.13	4.80
EC (dS/m)	0.23	0.20
OC (%)	0.67	1.32
P(kg/ha)	7.62	97.80
K (kg/ha)	523.66	467.00
Ca (mg/kg)	3762.66	327.00
Mg (mg/kg)	1489.5	105.67
S (mg/kg)	8.00	9.55
Fe (ppm)	18.11	14.72
Mn (ppm)	30.13	25.20
Cu (ppm)	37.43	5.62
Zn (ppm)	10.47	6.17
B (ppm)	0.58	0.14

Table 2: Nutrient management practices (Treatments) followed for the study

Treatment	Treatment details
T_1	Organic manure alone (Organic KAU-POP: FYM @ 25t/ha)
T_2	KAU-POP (N: P: K -100:50:50 kg/ha in the form of Urea, SSP, MOP + FYM @ 25t/ha
T_3	N: P: K - 100:75:75 in the form of Urea, SSP, MOP + FYM @ 25t/ha
T_4	N: P: K -100:50:50 kg/ha in the form of Factamphos (Basal), urea (top dress) and MOP +FYM @ 25t/ha
T_5	Soil test-based application of nutrients, in the form of Urea, SSP, MOP + FYM @ 25t/ha

Analytical procedure

Nitrate determination

Air dried plant sample was ground using pestle mortar and 0.4g of sample was weighed in 100ml of conical flask with 0.2g activated carbon and 40ml of 0.025 M of aluminum sulphate was added into the flask and shaken for 30 minutes. Solution was then passed through Whatman No 1 filter paper, then 0.2g of activated carbon was added to extracted solution again and repeated the extraction cycle. Extract was used for determination of nitrate (Cataldo *et al*., 1975) [9]. The readings were taken by spectrophotometer at a wavelength of 410 nm.

Oxalate analysis

Oxalate content was estimated by titration method. One gram of plant sample was taken in100ml conical flask. Seventy-five milliliters of 1.5M H_2SO_4 was added and shaken for 2 hours. Then the solution was passed through Whatman No 1 filter paper. Twenty-five ml of the filtrate was taken and titrated while hot against 0.05 M $KMnO_4$ solution until a faint pink color persisted for at least 30s.

Statistical analysis

Data produced from the experiment were subjected to statistical analysis by using wasp 2.0, software for factorial CRD (2x5x3).

Results and Discussion

Nitrates and oxalates content in amaranth cooked and raw samples

The data presented in (Table 3 and 4) indicate significant variations in the nitrate and oxalate content of cooked and raw amaranth after being steamed in tap water for four minutes. These variations were observed among different soil types and treatments as well as the interaction between soil types and treatments. Among the soil types, the lowest nitrate and oxalate content in both cooked and raw amaranth was observed in the lateritic soil, followed by the black soil. While considering the treatments, the cooked amaranth had the lowest nitrate content in T_1 (931.33mg/kg) compared to other treatments, T_2 (1793.66 mg/kg), T_3 (1714.66 mg/kg), T_4 (1342.00 mg/kg) and T_5 (1400.66 mg/kg). Similarly, the raw amaranth had the lowest nitrate content in T_1 (1408.00 mg/kg) but it was on par with T_5 (1576.66mg/kg), while significantly higher nitrate levels were recorded in the other treatments such as T_2 (1801.00 mg/kg), T_3 (1972.66mg/kg) and T_4 (1796.66mg/kg). The same trend was observed for oxalate content (Table 4) in cooked amaranth. In cooked amaranth, the lowest value of oxalate content was observed in T_1 (2301.00 mg/kg) which was on par with T_5 (2671.18 mg/kg) and in raw amaranth lowest value was noticed in T_1 (2881.28 mg/kg). The interaction between soil type and treatments was also significant and values ranged from 2130.94 to 3361.49 mg/kg. Lateritic soil registered significantly lower values compared to black soil. In the case of nitrate also, the interaction between soil type and nutrient management practices was significant. Nitrate content in cooked amaranth ranged from 498.66 to 2126.66 mg/kg, while in raw amaranth, it ranged from 1056.00 to 2229.33 mg/kg. In the cooked amaranth, T_1 (498.66mg/kg) in the lateritic soil showed the lowest value compared to all other treatments, and in the black soil, T_4 (1261.33 mg/kg) had the lowest value, which was on par with T_1 (1364.00 mg/kg).

According to the findings presented in the table 3 and 4, it is recommended to cook amaranth in order to reduce its nitrate (2.27 to 33.83%) and oxalate (11.20 to 20.13%) content.

Table 3: Nitrate content (mg/kg) in cooked and raw amaranth

	Nitrate content (mg/kg) in cooked sample			Nitrate content (mg/kg) in raw sample		
Treatment	**Black soil**	**Lateritic soil**	**Mean**	**Black soil**	**Lateritic soil**	**Mean**
T_1	1364.00	498.66	931.33	1760.00	1056.00	1408.00
T_2	2106.00	1481.33	1793.66	2174.66	1496.00	1835.33
T_3	2126.66	1302.66	1714.66	2229.33	1716.00	1972.66
T_4	1261.33	1422.66	1342.00	1613.33	1980.00	1796.66
T_5	1804.00	997.33	1400.66	1833.33	1320.00	1576.66
Mean	1732.40	1140.53		1922.133	1513.60	
A (CD at 5%)	228.90			153.19		
B (CD at 5%)	361.92			242.22		
A*B (CD at 5%)	511.84			342.55		

Table 4: Oxalate content in cooked and raw amaranth

	Oxalate content (mg/kg) in cooked sample			Oxalate content (mg/kg) in raw sample		
Treatment	**Black soil**	**Lateritic soil**	**Mean**	**Black soil**	**Lateritic soil**	**Mean**
T_1	2471.05	2130.94	2301	3121.386	2641.173	2881.280
T_2	3241.44	3061.36	3151.40	4055.120	3631.613	3843.366
T_3	3361.49	2641.17	3001.33	4021.786	3391.506	3706.646
T_4	3361.49	3061.36	3211.42	3571.586	3661.626	3616.606
T_5	2881.28	2461.09	2671.18	3481.546	2986.733	3234.140
Mean	3063.35	2671.18		3650.283	3262.536	
A (CD at 5%)	347.69			126.80		
B (CD at 5%)	549.75			200.49		
A*B (CD at 5%)	777.47			283.54		

Effect of treatments on amaranth yield

The data presented in (Table 5) indicates significant variations in amaranth yield among different soil types and treatments as well as their interaction. The lateritic soil demonstrated the highest yield value (19.99) which was comparable to the yield from black soil (17.23). Regarding the treatments, the one receiving NPK-100:50:50 in the form of Factamphos and MOP along with FYM-25t/ha -T_4 (23.62), exhibited a significantly higher yield. This treatment's yield was on par with T_2- KAU-POP NPK-100:50:50 with urea, SSP, MOP + lime based on soil test and T_3-NPK-100:70:75 with urea, SSP, MOP + FYM-25t/ha + lime based on soil test. The lowest yield was observed in T_1- organic KAU-POP (10.91g/plant). Nutrient application based on soil test - T_5 (16.50g/plant) gave yield similar to that of T_2 (21.44) and T_3 (20.57). From these results, it is evident that T_2, T_3, and T_4 and T_5 demonstrated higher yields by incorporating inorganic fertilizers with organic manure. Application of Factamphos (T_4) resulted in significantly higher yield than the other treatments involving urea and SSP as sources of N and P respectively (T_2, T_3 and T_5). This may be attributed to the reasons *viz*., (i) Factamphos contains 40 parts of ammonium phosphate and 60 parts of ammonium sulphate (20:20:0:13). Ammonium ion enhances P uptake by altering soil pH and phosphorus solubility (ii) About 85 to 95% of phosphorus in Factamphos is in the water-soluble form which has better mobility and easy penetrability as compared to superphosphate thus stimulating early root development and plant growth.

It is well known that inorganic fertilizer is a highly nutritious chemical, but excessive use of such fertilizers can result in elevated nitrate levels in vegetables, negatively impacting their quality (Wang *et al.*, 2007) [8].

Table 5: Effect of treatments on amaranth yield

Treatment	Yield (g/plant)			Reduction in the anti-nutrient content (%) after cooking (steaming)	
	Black soil	Lateritic soil	Mean	Nitrate (%)	Oxalate (%)
T_1	7.51	14.32	10.91	33.83	20.13
T_2	19.45	23.43	21.44	2.27	18.00
T_3	19.32	21.81	20.57	13.07	19.02
T_4	23.65	23.60	23.62	25.30	11.20
T_5	16.22	16.79	16.50	11.16	17.40
Mean	17.23	19.99			
A (CD at 5%)	3.36			Not statistically analysed	
B (CD at 5%)	5.31				
A*B (CD at 5%)	7.51				

Conclusion

The present study revealed that soil types as well as nutrient management practices have profound influence on the accumulation of nitrate and oxalate in amaranth. Organic manure alone treatment (Organic KAU-POP: FYM @ 25t/ha) is the best practice to reduce the anti-nutrient content. The anti-nutrient content decreased with cooking. Compared to black soil of Chittur, lateritic soil of Thrissur registered lower levels of nitrate and oxalate in amaranth. Among the integrated management practices, soil test-based application of nutrients + FYM-25 t/ha showed superiority over other treatments in reducing the contents of anti-nutrients in amaranth along with reasonably good crop yield.

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