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Evaluation of amaranthus cultivars for anti-nutritional factors at different leaf clipping stages

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Abstract
The study on "Evaluation of Amaranthus cultivars for anti-nutritional factors at different leaf clipping stages" was carried out at the experimental block of Department of Vegetable Science, College of Horticulture, Anantharajupeta, Railway Kodur, YSR Kadapa district, Andhra Pradesh during Spring-Summer season of 2021-22 with 15 amaranth cultivars collected from different regions. The experiment was laid out in a RBD with a factorial concept and comprised of 3 replications. The objective of this study was to determine the anti-nutrient content (oxalates, nitrates and tannins) of 15 amaranth cultivars at two clipping stages *i.e.,* at 25 DAS (Vegetative stage) and at flower initiation stage. The results showed significant variation in anti-nutrient content in the fifteen amaranth cultivars due to both cultivars and clipping stages. Anti-nutritional analysis revealed that lowest oxalate (191.30 mg/100 g) and nitrate content (109.80 mg/100 g) was recorded in Arka Varna while Arka Arunima recorded lowest tannin content (92.35 mg/100 g). In conclusion, as the plants grow older, there was an increased accumulation of anti-nutrients.

Keywords: Amaranth cultivars, clipping stages, anti-nutrients, oxalates, nitrates, tannins

Introduction
India, being blessed with a variety of natural surroundings and varying climates and seasons, has a number of species of edible leafy vegetables. Amaranthus is one of the India's most significant and well-liked green leafy vegetable, commonly known as "Thota Kura" in Telugu, belongs to the family Amaranthaceae. The Amaranthus genus has 50–60 species, some of which are wild species. They are grown for their leaves and grains. *A. tricolor, A. dubius, A. lividus, A. blitum, A. tristis,* and *A. viridis* are among the leafy amaranth species (2n = 34), whereas *A. cruentus, A. caudatus,* and *A. spinosus* are grain amaranthus species (2n = 32). It is the most popular leafy vegetable in south India cultivated in Kerala, Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, and Telangana states.
Among leafy vegetables, amaranthus have been generally reported to have excellent nutritional characteristics. This is due to the high content of vital micronutrients such as calcium, magnesium, iron, vitamin C and other essential nutrients such as gluten-free carbohydrate needed for healthy living (Achigan-Dako *et al.,* 2014; Jimoh *et al.,* 2018) [3, 8]. Vegetables including amaranth are an important component of the diet as they provide important nutrients and also have bioactive components such as antioxidants that help protect the body from long-term degenerated diseases (Raheena, 2007). However, the amaranth vegetable has also been shown to contain some anti-nutrients such as oxalates, nitrates tannins and phytic acid. These antinutrients bind nutrients and may reduce their bioavailability in the body (Abara, 2003; Agbaire & Emoyan, 2012) [1].
Various amaranth varieties contain different amounts of nutrients, anti-nutrient, and phytochemicals at different growth stages. There are newly developed varieties of amaranth with unknown nutrients, anti-nutrients and phytochemical contents. The objective of this study was therefore to investigate the presence of anti-nutritional factors at various leaf clipping stages so as to understand the extent to which they affect bioavailability of nutrients in the body.

Material and Methods
The present investigation was carried out at the experimental block of Department of Vegetable Science, College of Horticulture, Anantharajupeta, Railway Kodur, YSR Kadapa district, Andhra Pradesh during Spring-Summer season of 2021-22.

The experiment was laid in FRBD and replicated three times. The treatments comprised 15 different amaranth cultivars collected from different regions which includes released varieties, private and local varieties, with two clipping stages viz., the vegetative stage (25 DAS) and flower initiation stage.

Anti-Nutrient analysis

Determination of oxalates (mg/100 g): The oxalate content was determined from the modified titration procedure described by Unuofin *et al.* (2017) [14]. About 1 g of each of the pulverized plant samples was weighed in a conical flask and 75 mL of 3 M H_2SO_4 was added and agitated on a magnetic stirrer for one hour. The mixture was filtered and 25 mL of filtrate collected was heated to about 90 °C. The hot aliquot was titrated uninterruptedly against 0.05 M $KMnO_4$ till a light pink colour change which lasted for 15 s was observed. This marked the endpoint of the titration. The titre value of each of the plant sample extracts was multiplied by 2.2 mg of oxalate taken as the equivalence of 0.05 M of $KMnO_4$ used for titration.

Oxalate content = 2.2mg x titre value

Determination of nitrates (mg/100 g): The nitrate content in the test samples was determined by the calorimetric method using salicylic acid (Cataldo *et al.*, 1975) [6]. About 500 mg of fresh sample was weighed and put in a test tube and 10 mL of hot (90-95 °C) distilled water was added. The closed tubes were placed in a water bath at 80 °C for 30 minutes and shaken. The samples were cooled and centrifuged at 4500 rpm. The supernatant was decanted and weighed to determine the exact volume of extract. Chlorophyll in leaf extract was removed by adding 0.5 g $MgCO_3$ to the supernatant and centrifuged again. The supernatant containing the nitrate extract was treated with 2 N NaOH and a combination of Salicylic acid and H_2SO_4 in a ratio of 1:20. Standards were also prepared using sodium nitrate. Absorption was measured at 410 nm with a UV-Vis spectrophotometer.

Determination of tannins (mg/100 g): The tannins were determined by the Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared. Absorbance for test and standard solutions were measured against the blank at 700 nm with a UV-Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ 100g of dried sample.

Results and Discussion

Oxalates (mg/100 g): The data on oxalates content of different amaranthus cultivars as influenced by different leaf clipping stages and their interactions is presented in Table 1 and Fig.1 Significant variation was observed among the cultivars, clipping stages and their interactions.

Among the cultivars, Arka Varna(V_2) recorded significantly lowest oxalate content (191.30 mg/100g) which is statistically on par with Arka Arunima (V_3) (191.60mg/100g). However higher oxalate content (235.65 mg/100g) was recorded in Mysore Local (V_{13}).

Significant increase in the oxalate levels was observed as the plant matured. Oxalate content was significantly higher at second clipping (274.99) (C_2), while it was lowest at the first clipping stage (155.89 mg/100g) (C_1).

Among the interactions, significantly lower oxalates were found in Arka Arunima (134.80 mg/ 100 g) at the first clipping stage (C_1V_3) followed by CO-1(142.90 mg/100 g) (C_1V_6) at first clipping stage and maximum oxalate content was recorded in Mysore Local at flower initiation clipping stage (298.40 mg/100 g) (C_2V_{13}).

From these results it was observed higher oxalates concentration at flower initiation stage over vegetative stage. Variation in oxalate content might be due to cultivars, age of the plant, growing season, genetically based plant metabolism and environmental factors. The research findings were in conformity with Srivastava *et al.* (2002) [12], Anuja (2012a) [5], Nyonje *et al.* (2013) [10], Umar *et al.* (2011) [13], Hricova *et al.* (2021) [7] in amaranthus and Sethi *et al.* (2021) [11] in under-utilized vegetables. These results are also in accordance with the finding of Waldemar *et al.* (2005) that older plant had higher oxalates than the younger plant in dill. Another reason for this could be that the secondary plant substances (secondary metabolites) accumulate in tissues and organs during aging (Noggle and Fritz, 2006) [9]. The elevated levels of oxalates recorded at flower initiation stage of amaranthus cultivars is an indication to avoid the consumption at reproductive phase.

Nitrates (mg/100 g): The data pertaining to nitrates content in different amaranthus cultivars as influenced by two leaf clipping stages and their interactions is presented in Table 1 and Fig.2. Experimental results revealed that statistically significant variation found with respect to nitrate content among the amaranthus cultivars. The lower nitrate content (109.80 mg/100 g) was recorded in Arka Varna (V_2) which stood statistically on par with Arka Arunima (111.0 mg/100 g) and highest nitrate content (138.90 mg/100 g) was recorded in Tirupathi Local (V_{15}).

Nitrate content varied significantly at both the vegetative and flower initiation stages. There was significant decrease in the mean nitrate content from 153.49 mg/100 g at vegetative stage (C_1) to 94.83 mg/100 g at flower initiation stage (C_2).

With respect to the interaction effect, a significantly lower nitrates (81.20 mg/100 g) was recorded in Arka varna at flower initiation stage (C_2V_2) which was statistically on par with Arka Arunima (84.40 mg/100 g) (C_2V_3) and Arka Suguna (84.60 mg/100 g) (C_2V_1) at second clipping stage and higher nitrate content (167.20 mg/100 g) was recorded in Tirupathi Local at vegetative stage (C_1V_{15}).

The results showed that nitrate content decreased with plant maturity. The amount of nitrate content in plants is determined mainly from its genetically based metabolism, the age of the plant, environmental factors, the amount of available nitrate in the soil, significant negative correlation between nitrate content in the plant and nitrate reductase activity (Anjana *et al*., 2007) and translocation of secondary metabolites from leaves to flowering parts (Noggle and Fritz, 2006) [9]. The decreasing trend in nitrate content in vegetables with maturity was also reported by Musa *et al.* (2011) who found that nitrate content of *A. cruentus* decreased with plant maturity. The results are also in accordance with Srivastava *et al.* (2002) [12], Umar *et al.* (2011) [13], Anuja (2012) [5], Nyonje *et al.* (2014) [10] in amaranthus and Sethi *et al.* (2021) [11] in under-utilized vegetables.

Tannins (mg/100 g): The data pertaining to tannin content in different amaranthus cultivars as influenced by two leaf clipping stages and their interactions is presented in Table 1 and Fig.3. Significant variation was found among the amaranthus cultivars regarding tannin content. Lowest tannin content (92.35 mg/100 g) was found in Arka Arunima (V_3) which was statistically on par with Pusa Kiran (V_5) (93.40 mg/100 g) and CO-2 (V_7) (94.50 mg/100 g) and highest tannin content (110.95 mg/100 g) was recorded in Kadapa Local Green (V_{11}).

There was a significant variation in tannin content due to different clipping stages. The means of tannin content were 87.41 mg/100 g and 114.89 mg/100 g at vegetative stages (C_1) and at flower initiation (C_2) stage indicating that tannin content was increased gradually as the plant matures.

There was a significant variation observed among the interaction effect of variety and clipping stages with respect to tannin content. The minimum tannin content (90.60 mg/100 g) was found from the treatment combination of Arka Arunima at the vegetative stage (C_1V_3) and the maximum (125.70 mg/100 g) was found from the treatment combination of Kadapa Local Green at flower initiation stage (C_2V_{11}).

These findings from the study revealed that the tannin content varied significantly and showed increasing trend towards plant maturity. This might be due to varietal difference, age of the plant, genetically based plant metabolism and environmental factors. These results are in conformity with those of Nyonje *et al.* (2014) [10] in amaranthus, Sethi *et al.* (2021) [11] and Abdi *et al.* (2022) [2] in under-utilized leafy vegetables.

Table 1: Influence of stage of clipping on oxalate, nitrate and tannin content in different amaranth cultivars.

Cultivars	Oxalates (mg/100 g)			Nitrates (mg/100 g)			Tannins (mg/100 g)		
	Clipping stages			Clipping stages			Clipping stages		
	C1	C2	Mean	C1	C2	Mean	C1	C2	Mean
V_1-Arka Suguna	161.20	264.60	212.90	145.20	84.60	114.90	81.90	108.70	95.30
V_2-Arka varna	148.40	234.20	191.30	138.40	81.20	109.80	91.80	116.30	104.05
V_3-Arka Arunima	134.80	248.40	191.60	138.60	83.40	111.00	80.60	104.10	92.35
V_4-Pusa Lal Choulai	154.70	284.90	219.80	147.80	91.80	119.80	90.40	112.30	101.35
V_5-Pusa Kiran	149.50	264.70	207.10	149.90	97.40	123.65	83.70	103.10	93.40
V_6-Co-1	142.90	259.40	201.15	139.40	89.10	114.25	84.20	107.80	96.00
V_7-Co-2	148.30	278.50	213.40	141.20	89.80	115.50	82.10	106.90	94.50
V_8-Arun	152.80	269.40	211.10	158.40	91.20	124.80	84.10	116.30	100.20
V_9-Pattucheera	156.90	271.20	214.05	157.50	93.40	125.45	83.40	118.40	100.90
V_{10}-FH-70	151.90	291.30	221.60	161.40	97.30	129.35	86.40	115.40	100.90
V_{11}-Kadapa Local Green	168.70	287.50	228.10	164.80	101.40	133.10	96.20	125.70	110.95
V_{12}-Kadapa Local Red	170.80	291.40	231.10	164.30	106.90	135.60	94.20	124.30	109.25
V_{13}-Mysore Local	172.90	298.40	235.65	165.80	104.60	135.20	93.40	121.80	107.60
V_{14}-Sirraku	159.40	296.70	228.05	162.40	99.80	131.10	86.70	119.90	103.30
V_{15}-Tirupathi Local	165.20	284.30	224.75	167.20	110.60	138.90	92.10	122.40	107.25
Mean	155.89	274.99		153.49	94.83		87.41	114.89	
Factors	V	C	V X C	V	C	V X C	V	C	V X C
CD at 5 %	6.041	2.206	8.543	3.984	1.455	5.634	3.356	1.226	4.747
SE (m) ±	2.128	0.777	3.010	1.404	0.513	1.985	1.183	0.432	1.672

C_1- Clipping at vegetative stage (25 DAS); C_2- Clipping at flower initiation stage

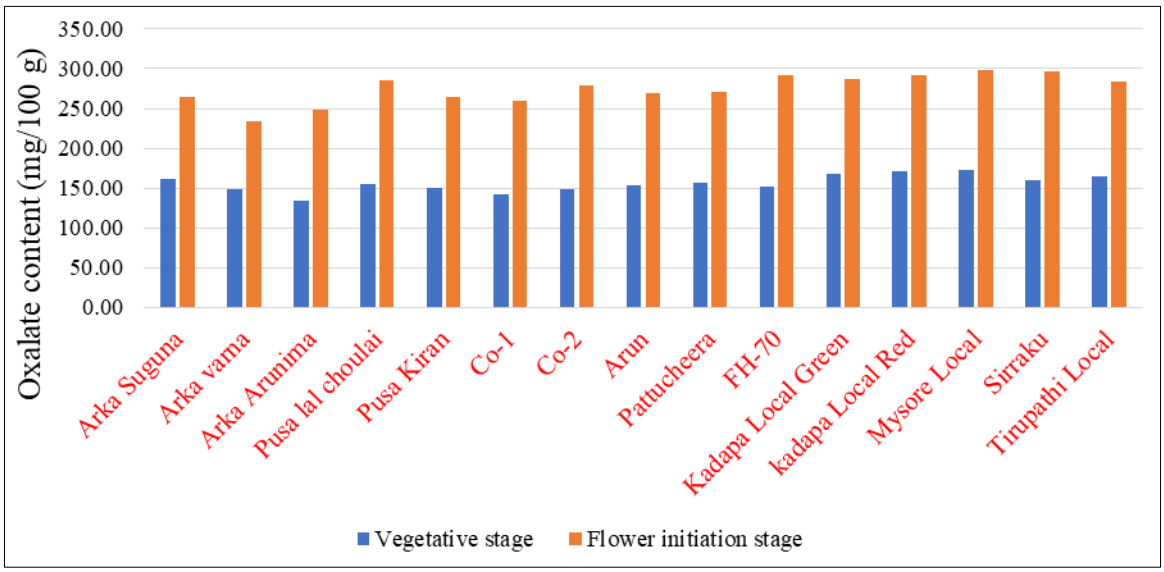

Fig 1: Impact of stage of clipping on oxalate content (mg/100 g) in different amaranth cultivars.

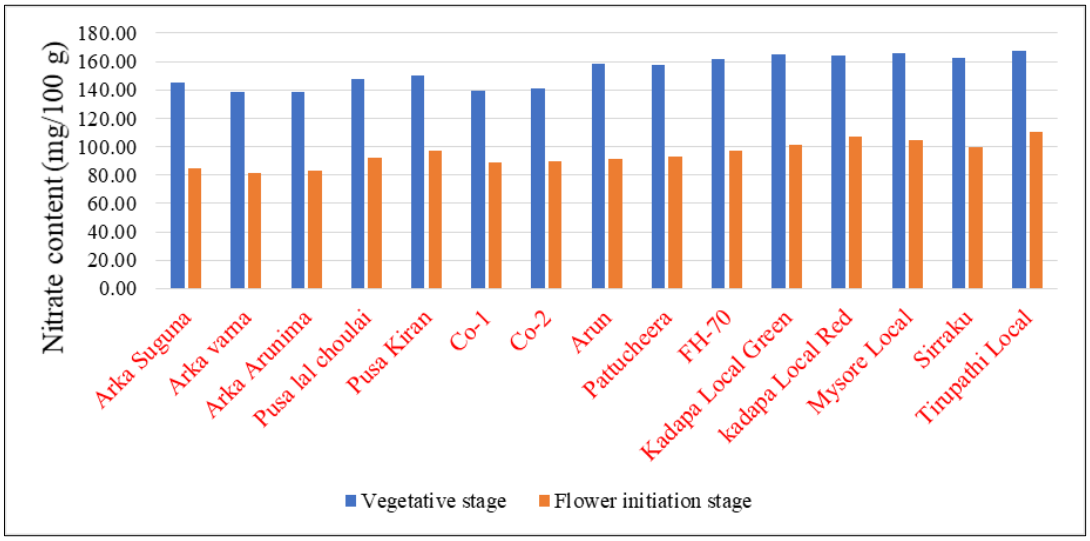

Fig 2. Impact of stage of clipping on nitrate content (mg/100 g) in different amaranth cultivars

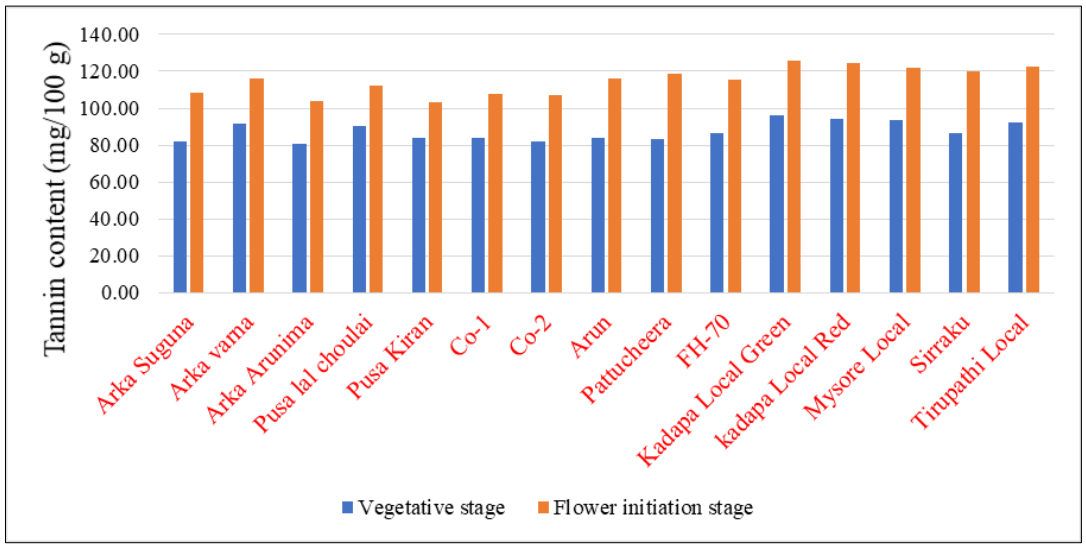

Fig 3: Impact of stage of clipping on tannin content (mg/100 g) in different amaranth cultivars

Conclusion

There were significant differences in the anti-nutrient content among the cultivars at different clipping stages. The anti-nutrient content increased with maturity from vegetative stage to flower initiation stage. Amaranth leaves should be consumed before flowering as they have generally lower anti-nutrients. Selection of the cultivars can be done according to their anti-nutrient. Arka Varna recorded lowest oxalate and nitrate while Arka Arunima recorded lowest tannin content among the studied cultivars.

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