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Effects of Nitrogen Fertilizer Rates and Seasons on Polyphenols and Catechins of Non-aerated Green Tea Processed from Seedling Tea (*Camellia sinensis*)

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ABSTRACT

Seasonal biochemical profiling was performed on non-aerated green tea processed from high nitrogenous fertilizer rates (0-800 kg N/ha/year) trial on seedling tea to determine the effects of fertilizer rates and seasons on total polyphenols, catechins and caffeine levels. Increasing fertilizer rates significantly, ($p \leq 0.05$) increased total polyphenols and catechins levels up to 400 kg N/ha/year rate. There was no increase in the polyphenolic compounds beyond this rate. Although some non-aerated green tea producing countries apply very high rates, of up to 800 kg N/ha/year, to improve quality, in Kenyan seedling tea, there were no green tea quality benefits from applying higher rates beyond 400 kg N/ha/year. Polyphenols levels ranged from 21.19-21.85% while catechins ranged ~~catechin~~ form 16.01-17.31% between 0-800 kg N/ha/year application rates. There were seasonal biochemical variations. Polyphenols levels ranged from 22.91-23.37% during October-December, 21.17-21.18% in January-March, 16.47-16.48% in April-June and 20.38-20.52% in July–September. Catechins values were 17.32-16.05%, 17.66-17.23%, 14.91-15.73% and 17.39-17.59%, while caffeine values were 3.02-3.20%, 3.43-3.25%, 3.88-3.94%, and 2.70-2.78% in the respective seasons. Total polyphenols were higher during the cold and wet (October-December) and hot and wet (April-June) seasons and low in cold and dry (July September) and hot and dry (January-March) seasons. Irrespective of the temperatures, wet months produced higher levels of the parameters evaluated.

INTRODUCTION

Tea beverages are processed from fresh leaves of *Camellia sinensis*. Kenya and most African countries predominantly produce black Cut, Tear and Curl (CTC) tea, mainly for the export market (*1*). Lack of serious product diversification of tea products and over reliance on black CTC tea has led to unstable earnings for the industry, due to the over supply of black tea in the market and stagnant/declining tea prices. Farmers therefore realize low returns causing industry dissatisfaction. Research to develop alternative tea products to avail to the world market alternative products and enhance tea revenue has therefore become a priority (*2, 3*). Non-aerated (green) tea is one such product with a potential for good market returns in China, Japan and other countries. Green tea has been particularly attractive to the health conscious tea consumers (*4-7*). The tea products have been used to manage many ailments in the Asian continent (*8*), and extensive health benefits of tea have been subjects of ancient Chinese writing (*9, 10*). Apart from use as a beverage, non-aerated green tea has also become the raw material for extracts to use in various health foods, dietary supplements, and cosmetics (*11*). The market segment for non-aerated green tea has recently expanded because of cultural interaction and health related reasons. Non-aerated green tea is made from fresh leaves of *Camellia sinensis* that have undergone minimal oxidation during processing (*12, 13*). Studies on the development of non-aerated green tea from seedling and clonal teas have not been undertaken for the Kenyan tea.

The constituents, which influence the taste and colour of tea, are mainly polyphenolic compounds, and caffeine. Young tea leaves contain 23-26% dry matter, of most of which are catechins, and anthocyanins (*14, 15*). The catechins (flavan-3-ols) constitute up to 30% of the dry weight (*16*). The dominant catechins in tea leaves are (+)-catechin (C), (+)-gallocatechin (GC), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG). Related compounds, (-)-epicatechin-3,5-digallate and the 3-methyl gallates of (-)-epicatechin and (-)-epigallocatechin have been reported in fresh green leaf (*14*). These catechins largely determine the quality of non-aerated green tea (*17, 18*). Climatic (*15, 19-*

23) and agronomic (*22-24*) factors influence the flavanol content of the fresh leaf and processed teas. Tea harvesting removes high amounts of nutrients with crop (*25, 26*). To sustain production, regular fertilizer application is necessary. For aerated black tea in Kenya, 150-200 kgN/ha/year is recommended black tea production (*27*). In non-aerated green tea production, use of up to 800 kg N/ha/year is applied in tea fields in Japan to improve yields and quality (*28*). The influence of rates of nitrogen on quality of non-aerated green tea has not been evaluated under tropical conditions like in Kenya. This paper reports the influence of seasons and nitrogenous fertilizer rates on quality of non-aerated green seedling teas in Kenya.

MATERIALS AND METHODS

Site and materials

Leaf for the trial was obtained from seedling teas planted in 1959 at Timbilil (0°22'S; 35° 21'E; 2,180 m above mean sea level (amsl)). The long term fertilizer trial was set to establish the effects of high rates and splitting annual application of nitrogenous fertilizer on tea in 1983 (*29*) at Timbilil Estate of the Kenya Agricultural and Livestock Research Organization, Tea Research Institute (KALRO-TRI) Kericho, Kenya. The experiment was originally set as a factorial two trial with rates and splitting annual applications as the treatments. However, after 15 years of experimentation without response to splitting annual nitrogen application, it was converted to long term trial on high rates of nitrogen downgraded to test long term effects of high rates of nitrogen only in randomized complete block design (RCBD), replicated three times. The fertilizer rates were 0, 50, 100, 200, 400, and 800 kg N/ha/year applied as NPKS 25:5:5:5 in October every year.

Leaves for green tea processing was obtained from October to September for 2 years (2011-2012 and 2012-2013). Plucking was done in 4 seasons of each year in a 10-14 day interval per plucking round. The seasons were divided into cold and dry (July-September) warm and wet (October to December, warm and dry (January-March) and cold and wet (March-May) (*30*).

Non-aerated green tea processing

About 500g per plot of two leaves and a bud of fresh green leaf, were hand plucked from the seedling tea bushes in the four seasons each year for processing. The leaves were kept at ambient temperatures and transported to a miniature processing factory where they were processed into non-aerated green tea as outlined in an earlier study (*3*). The leaves were steamed for 1 min (Philips model HD 9120, China), macerated using a CTC machine, 3 cuts (Tea Craft limited, Bedford, UK) and dried at 120ºC in a fluidized bed drier (Tea Craft limited, Bedford UK) to a moisture content (MC) of 4%. Processed teas were stored in sealed silver lined sachets at room temperature (20-24°C) to await biochemical analyses.

Biochemical assays

Extraction and analysis of total polyphenols was done according to the procedure by International Standards Organization (*31*). Ground tea sample (0.2g) was weighed into a graduated extraction tube and 5mL of 70% hot methanol/water v/v added, stoppered and mixed under vortex. Incubation followed at 70°C for 10 min (water bath) with vortexing at 0, 5 and 10min, cooling to room temperature and then centrifuging at 3000rpm for 10min. A second extraction was done; the extracts combined and made up to 10mL with cold methanol/water v/v at 70%.

Following the extraction, further dilution was done by transferring 1.0 ml of extract into 100 ml volumetric flask and by adding distilled water to the mark. Gallic acid (GA) standard solutions were prepared by transferring 1, 2, 3, 4 and 5 ml solutions corresponding to 10, 20, 30, 40 and 50μg anhydrous GA, into 100 ml volumetric flasks and filled to the mark with distilled water. One (1 ml) of the diluted tea samples and gallic acid standards were transferred into separate tubes and 5 ml Folin-Ciocalteau phenol reagent added to each tube and mixed by vortexing (Rotamixer, Huck and Tucker, England). Within 5 min of addition of Folin-Ciocalteau phenol reagent, 4.0 ml of sodium carbonate solution was added into each tube, mixed again and allowed to stand for 60 min at room temperature (24°C). Optical densities of the samples and standards were measured in a 1cm cuvette cell in a spectrophotometer at 765nm wavelength (Model CE 393 digital grating Spectrophotometer series 2).

Mass of anhydrous GA in the 1.0 ml aliquots of the standard solutions were calculated using the formula: $m = (m_o \times V \times w_{DMstd} \times 10000)/(100 \times 100)$. Where; m_o was the mass of GA monohydrate (g) used to prepare the stock standard solution; V was the volume of GA stock standard solution (g) used to prepare the standard solutions; w_{DMstd} was the dry matter content, expressed as a mass fraction (%) of the GA. A best-fit linear calibration curve from the mass of anhydrous GA standards was constructed against the GA standard optical densities. The total polyphenol (TP) content was expressed as a percentage by mass on a sample dry matter (DM) basis and calculated using the formula:

wT = ((Dsample – D intercept) x Vsample x d x 100)/ (Sstd x m sample x 10000 x wDM, sample).

Where; D sample = optical density of the sample test solution; D intercept = optical density at the point of the best-fit linear calibration line intercepts the y-axis; Sstd =was the slope of the best fit linear calibration; m sample = mass (g) of the sample test portion; Vsample = sample extraction volume in ml (10 ml for leaf tea); d = dilution factor used prior to the colorimetric determination and wDMsample = % dry matter content of the test sample.

The extraction and analysis of catechins was done according to the procedures by International Standards Organization (*32*). Ground tea sample (0.2g) was weighed into a graduated extraction tube and 5mL of 70% hot methanol/water v/v added, stoppered and mixed under vortex. Incubation followed at 70°C for 10 min (water bath) with vortexing at 0, 5 and 10min, cooling to room temperature (24 C°) and then centrifuging at 3000rpm for 10min. A second extraction was done, the extracts combined and made up to 10mL with cold methanol/water v/v at 70%.

High performance liquid chromatography (HPLC) analysis was done according to the ISO procedure (*32*). Approximately one (1) ml of sample was pipetted into a test tube and diluted to 5mL with a stabilizing solution (10% v/v acetonitrile with 500µg/ml of EDTA and 500µg/ml ascorbic acid), filtered and loaded into 2mL vials. A Shimadzu LC 20 AT HPLC fitted with a SPD-20 UV-Visible detector and C_6, 25cm x 4.6 i.d. column was used for determination at 278nm. The solvent systems for gradient elution included: Mobile phase A (9:2:89 v/v/v Acetonitrile: Acetic acid: EDTA) and mobile phase B (80:2:18 v/v/v Acetonitrile: acetic acid: EDTA) at a flow rate of 1mL/min. The column temperature was set at 35±0.5°C and the injection volume of 20µL was used. The conditions for the binary gradient was set as follows; 100% solvent A for 10 min then over 15 min a linear gradient to 68% mobile phase A, 32% mobile phase B and held at this composition for 10 min. The conditions were again reset to 100% mobile phase A and allowed to equilibrate for 10 min before the next injection.

Catechin identification was done by comparing the retention times of samples and standards under similar conditions. The standards used were Catechin (+)C, Epigallocatechin (-)EGC, Epicatechin (-)EC, Epigallocatechin gallate (-)EGCG and Epicatechin gallate(-)ECG. Total catechins (TC) were expressed as a percentage by mass on a sample dry matter basis and given as a summation of individual catechins as; percentage Total Catechin= [(%EGC) + (%+C) + (%EC) + (%EGCG) + (%ECG)].

About 2g of well-mixed sample was accurately weighed into a moisture dish and transferred to a hot-air-oven previously heated to temperatures of 130°C and drying done for 1h (Mermert Model UL 50, Germany). The final weight of the sample was taken after drying and cooling in a desiccator (Determination of Moisture, AOAC, 2012). The sample residue was reported as total solids and loss in weight as moisture.

$$\%\,\text{Moisture} = \frac{(\text{Wt of sample before drying} - \text{Wt of sample after drying} \times 100)}{\text{Wt of sample before drying}}$$

The obtained data was subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Effects of nitrogenous (N) fertilizer rates and season on non-aerated green tea quality parameters.

The year one of the research started in October 2011 and ended in September 2012, while year two started in October 2012 and ended in September 2013. The time periods covered replicated all the four seasons experienced in Kenyan tea growing regions (*30*). The four seasons included warm and dry (January to March), cold and wet (April to June), cold and dry (July to September) and warm and wet (October to December). The seasons reported in this research included one more distinct season between the month of April to September which slightly differed from those reported by Ng'etich et al., (33) that had only three.

The catechins, total polyphenols and caffeine changes with nitrogen rates in the two years (2011-2012 and 2012-2013), are presented in (Table 1). There were significant differences ($p \leq 0.05$) in the quantities of the compounds with nitrogen rates during the two years and between the months in the years. The between the years variations implied that environmental factors (*30*) controlling growth and production of the parameters (*33*) including rainfall and temperatures were not uniform in the two years. This made it necessary to interpret results from the two different years independently.

There was an increase in catechins and total polyphenols content of non-aerated green tea with the increase in nitrogen rates between 0 and 400 kg N/ha/year (Table 1) in the two years. However, the levels of these parameters were not significantly ($p \leq 0.05$) different between the 400 and 800 kg N/ha/year rates. Unlike the improvement in these parameters with high rates of nitrogen observed in Japan (*28*), these results demonstrate that for Kenya, in the production of non-aerated green tea from seedling tea, there

Table 1: Effects of varying nitrogen fertilizer rates on non-aerated green tea parameters (%) during 2011-2012 and 2012-2013

Year	N-Rates	GA	EGC	CAT	CAF	EC	EGCG	ECG	TC	PLY
2011-2012	0-800 kg	0.51±0.12	6.02±0.96	0.44±0.30	3.31±0.61	1.31±0.32	6.68±1.05	2.37±0.49	16.80±1.73	20.30±2.61
2012-2013	0-800 kg	0.44±0.08	6.39±0.78	0.50±0.19	3.06±0.38	1.78±2.28	6.82±0.69	2.34±0.34	17.52±1.27	21.48±1.38
	Mean	0.47	6.21	0.47	3.19	1.54	6.75	2.35	17.16	20.89
	LSD, (p=0.05)	0.02	0.13	0.05	0.08	0.29	0.15	0.08	0.27	0.31
	CV (%)	17.71	11.39	51.55	12.42	100	11.94	17.03	8.15	7.74
2011-2012	0kg N	0.47±0.08	5.77±1.10	0.38±0.13	3.01±0.34	1.05±0.35	6.05±1.06	2.14±0.60	16.01±2.83	19.72±2.69
	50 kg N	0.47±0.10	5.80±0.99	0.40±0.14	3.05±0.41	1.27±0.21	6.19±1.14	2.32±0.64	16.60±1.69	19.99±2.58
	100 kg N	0.49±0.15	6.02±0.89	0.42±0.14	3.38±0.67	1.34±0.32	6.86±0.78	2.36±0.35	16.74±1.06	20.29±2.76
	200 kg N	0.53±0.12	6.04±0.76	0.44±0.19	3.40±0.67	1.35±0.22	6.97±0.79	2.38±0.31	16.83±1.24	20.49±2.82
	400 kg N	0.55±0.12	6.12±1.04	0.45±0.15	3.51±0.75	1.40±0.22	6.99±1.06	2.41±0.30	17.20±1.28	20.50±2.39
	800 kg N	0.54±0.10	6.37±0.87	0.54±0.66	3.49±0.52	1.44±0.39	7.00±0.97	2.59±0.54	17.31±1.51	20.81±2.43
	Mean	0.51	6.02	0.44	3.31	1.31	6.68	2.37	16.79	20.3
	LSD, (p=0.05)	0.04	0.32	0.14	0.18	0.12	0.33	0.2	0.68	0.45
	CV (%)	15.75	11.42	16.65	11.99	19.67	10.61	17.98	8.67	4.73
2012-2013	0kg N	0.41±0.06	6.05±0.92	0.44±0.13	2.84±0.32	1.22±0.47	6.65±0.61	2.20±0.52	17.20±1.45	21.19±1.19
	50 kg N	0.42±0.09	6.32±0.67	0.45±0.18	2.87±0.35	1.34±0.42	6.75±0.65	2.26±0.24	17.40±1.17	21.29±1.41
	100 kg N	0.43±0.06	6.34±0.78	0.45±0.18	2.99±0.46	1.51±0.20	6.77±0.82	2.34±0.16	17.49±1.40	21.42±1.22
	200 kg N	0.45±0.08	6.46±0.71	0.48±0.19	3.18±0.31	1.52±0.35	6.86±0.63	2.37±0.44	17.58±1.14	21.54±0.94
	400 kg N	0.45±0.09	6.55±0.63	0.57±0.19	3.21±0.23	1.59±0.36	6.93±0.53	2.40±0.23	17.59±1.08	21.60±1.28
	800 kg N	0.47±0.08	6.62±0.84	0.57±0.21	3.26±0.38	3.46±5.25	6.95±0.83	2.46±0.23	17.86±1.29	21.85±2.01
	Mean	0.44	6.39	0.49	3.06	1.78	6.82	2.34	17.52	21.48
	LSD, (p=0.05)	0.03	0.3	0.08	0.14	0.96	0.27	0.15	0.52	0.39
	CV (%)	15.85	9.93	32.59	9.73	11.58	8.53	13.55	6.42	3.95

Value =Mean ± S.D. CV= Coefficient of variation. (%). GA=Gallic acid. EGC=Epigallocatechin. C=Catechin. CAF=Caffeine. EC=Epicatechin. EGCG=Epigallocatechingallate. ECG=Epicatechin gallate. TC=Total Catechin. PLY=Polyphenols.

is no quality benefit in applying beyond 400 kg N/ha/year. Caffeine also significantly ($p \leq 0.05$) increased with the increase in nitrogen rates as had been observed previously (*34-36*). The polyphenols and catechins results were in contrast to production of aerated black tea where tea yields can be increased by application of nitrogenous fertilizers up to a maximum of 470 kg N/ha/year in Kenya (*37*) and Sri Lanka (*38*), although the economic rates lie between 100-220 kg made tea (mt)/ha/year (*27, 39*) in Kenya.

Effects of season and time of the year on non-aerated green tea quality parameters

The changes in the non-aerated green tea quality parameters with respect to the time of the year are presented in Table 2. Detailed seasonal variations at the different rates of nitrogen are presented in Figures 1 and 2. There were significant seasonal variations in the parameters. The patterns of monthly variations (Figures 1 and 2) did not change with nitrogen fertilizer rates. The total polyphenols and individual catechins levels were higher during the warm and wet (October-December), warm and dry (January-March)~~, and hot~~ cold and wet (April-June) seasons and low in cold and dry (July September) seasons in both years. Indeed, the observed increase in the parameters with rates of nitrogen (Table 1) was observed on monthly basis (Figures 1 and 2). The rainfall and mean temperatures recorded during

Table 2: Effects of varying nitrogen rates on quality of non-aerated green tea Year1 (2011-2012) and 2(2012-2013)

Year	Monthly	GA	EGC	C	CAF	EC	EGCG	ECG	TC	PLY
2011-2012	Oct	0.52±0.06	5.70±0.28	0.46±0.05	3.02±0.32	1.40±0.22	7.26±0.50	2.47±0.16	17.32±0.88	22.91±1.41
	Nov	0.64±0.07	5.15±0.43	0.33±0.05	3.59±0.28	1.37±0.31	8.25±0.61	2.85±0.32	17.94±1.23	22.99±0.85
	Dec	0.58±0.07	4.58±0.40	0.30±0.04	3.20±0.30	1.27±0.14	7.36±0.62	2.54±0.28	16.05±1.16	23.37±1.26
	Jan	0.40±0.11	5.78±0.46	0.40±0.22	3.43±0.38	1.65±0.35	7.17±0.64	2.66±0.32	17.66±1.30	21.17±0.62
	Feb	0.58±0.04	6.58±0.86	0.31±0.25	3.26±0.43	1.23±0.12	6.56±0.52	2.25±0.14	16.92±0.86	21.19±0.59
	Mar	0.57±0.04	6.58±0.79	0.28±0.22	3.25±0.49	1.35±0.23	6.73±0.57	2.29±0.19	17.23±0.87	21.18±0.60
	Apr	0.53±0.14	5.87±1.01	0.52±0.61	3.88±0.73	1.10±0.17	6.02±1.13	2.18±0.69	14.91±3.50	16.47±1.20
	May	0.53±0.14	5.87±1.01	0.52±0.62	3.88±0.72	1.10±0.17	6.02±1.14	2.18±0.70	15.68±0.86	16.47±1.21
	Jun	0.54±0.09	5.92±0.96	0.49±0.33	3.94±0.56	1.10±0.14	6.10±0.83	2.12±0.62	15.73±0.67	16.48±1.06
	July	0.41±0.08	6.71±0.64	0.54±0.08	2.70±0.26	1.40±0.38	6.27±0.99	2.30±0.54	17.39±1.58	20.38±0.95
	Aug	0.14±0.06	6.67±0.59	0.54±0.09	2.70±0.24	1.34±0.41	6.10±0.90	2.29±0.60	17.12±1.56	20.44±1.02
	Sep	0.41±0.05	6.86±0.56	0.56±0.07	2.78±0.18	1.38±0.48	6.27±1.01	2.27±0.33	17.59±1.39	20.52±1.02
	Mean	0.51	6.02	0.44	3.31	1.31	6.68	2.37	16.79	20.3
	LSD, (p=0.05)	0.05	0.45	0.19	0.26	0.17	0.47	0.28	0.96	0.63
	CV (%)	15.75	11.42	66.36	11.99	19.67	10.61	17.98	8.67	4.73
2012-2013	Oct	0.45±0.07	6.69±0.73	0.51±0.17	2.92±0.48	1.46±0.35	6.33±0.91	2.16±0.34	17.15±1.83	20.33±0.85
	Nov	0.51±0.06	3.37±0.78	0.23±0.16	3.04±0.34	1.36±0.20	6.62±0.72	2.13±0.74	16.80±1.18	20.00±0.59
	Dec	0.45±0.05	5.90±0.37	0.42±0.11	2.96±0.32	1.14±0.11	6.33±0.42	2.24±0.18	16.09±0.82	20.19±0.63
	Jan	0.44±0.12	6.18±0.74	0.55±0.26	3.34±0.42	1.29±0.57	7.01±0.86	2.50±0.30	17.57±1.44	22.35±1.59
	Feb	0.45±0.08	6.29±0.71	0.54±0.26	3.35±0.37	1.41±0.60	7.07±0.76	2.52±0.29	17.91±1.31	23.09±0.67
	Mar	0.46±0.06	6.36±0.64	0.49±0.23	3.34±0.25	1.52±0.56	7.19±0.39	2.50±0.20	18.11±0.95	23.20±0.63
	Apr	0.45±0.12	5.69±1.01	0.45±0.18	3.09±0.27	1.36±0.36	7.34±0.49	2.34±0.54	17.18±1.23	20.91±0.62
	May	0.39±0.07	6.10±0.87	0.51±0.15	3.06±0.23	1.48±0.24	7.22±0.45	2.36±0.11	17.67±0.96	20.80±0.54
	Jun	0.39±0.05	6.10±0.44	0.55±0.13	3.12±0.19	1.49±0.18	7.28±0.49	2.33±0.10	17.73±0.78	20.64±0.42
	July	0.43±0.05	6.85±0.57	0.56±0.12	2.88±0.37	1.58±0.50	6.43±0.43	2.31±0.13	17.71±1.13	21.64±1.54
	Aug	0.43±0.04	7.06±0.44	0.55±0.08	2.84±0.34	3.23±4.83	6.48±0.39	2.33±0.11	18.11±0.92	22.28±0.69
	Sep	0.43±0.04	7.07±0.36	0.58±0.07	2.81±0.37	3.99±5.64	5.51±0.31	2.33±0.12	18.18±0.70	22.36±0.68
	Mean	0.44	6.39	0.49	3.06	1.78	6.82	2.34	17.52	21.48
	LSD, (p=0.05)	0.01	0.42	0.11	0.2	1.35	0.38	0.21	0.74	0.56
	CV (%)	15.85	9.93	32.59	9.73	115.83	8.53	13.55	6.42	3.95

Value =Mean ± S.D., CV= Coefficient of variation. The values are mean value rates for 0, 50, 100, 200, 400 and 800kgN/ha/yr. (%). GA=Gallic acid. EGC=Epigallocatechin. C=Catechin. CAF=Caffeine. EC=Epicatechin. EGCG=Epigallocatechingallate. ECG=Epicatechin gallate. TC=Total Catechin. PLY=Polyphenols. Values presented in the tables are in percentage (%)

the study period is presented in Table 3. Wet periods were conducive for production of the quality parameters (Figures 3 and 4). Temperature variations also had a large influence on the parameters (Figures 5 and 6). However, the variations in the parameters observed in this study were much smaller than those observed in Japan (*28*). Variations of the same low magnitudes had been observed in aerated black tea in Kenya (*36*). Caffeine also showed seasonal variations as had been observed in previous studies (*36, 40*). The pattern of seasonal variations did not change with nitrogen fertilizer rates.

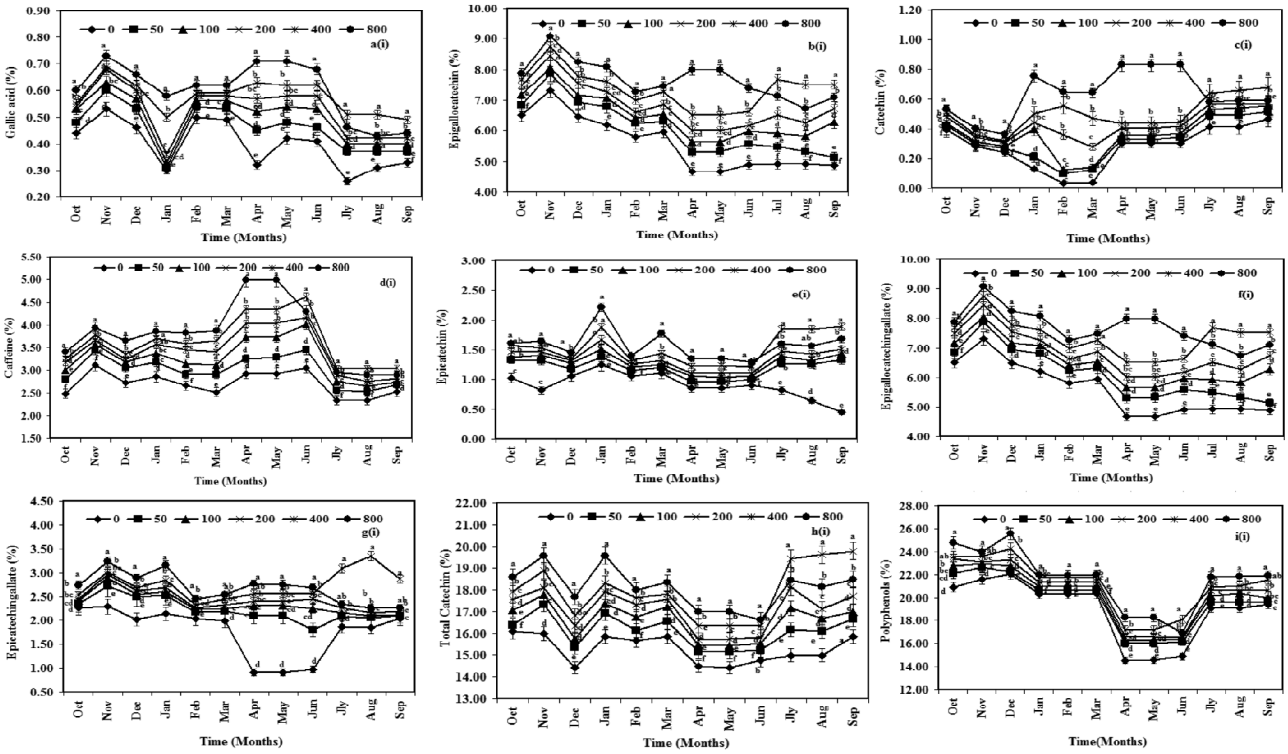

Fig. 1: Catechin profile of non-aerated green tea processed from seedling tea cultivated with varying rates of Nitrogen (kgN) in year 1 (2011-2012) October to September.

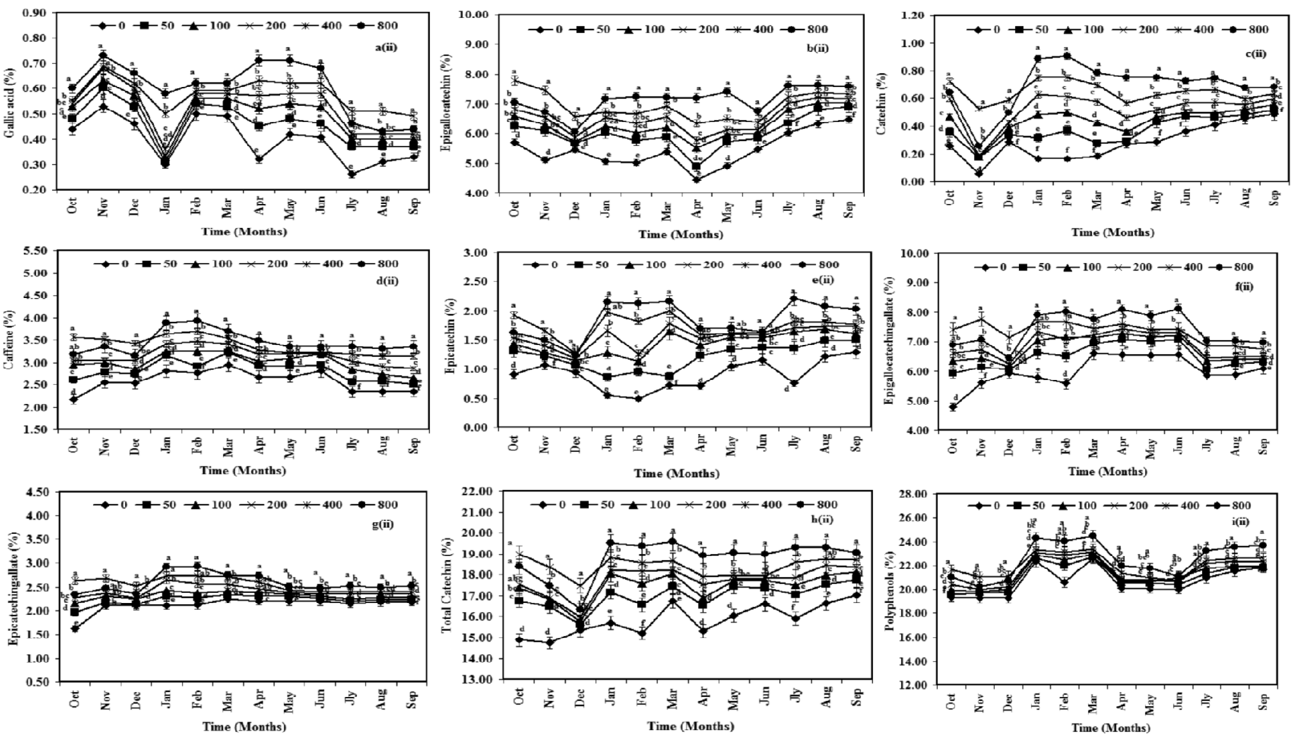

Fig. 2: Catechin profile of non-aerated green tea processed from seedling tea cultivated with varying rates of Nitrogen (kgN) in year 2 (2012-2013) October to September.

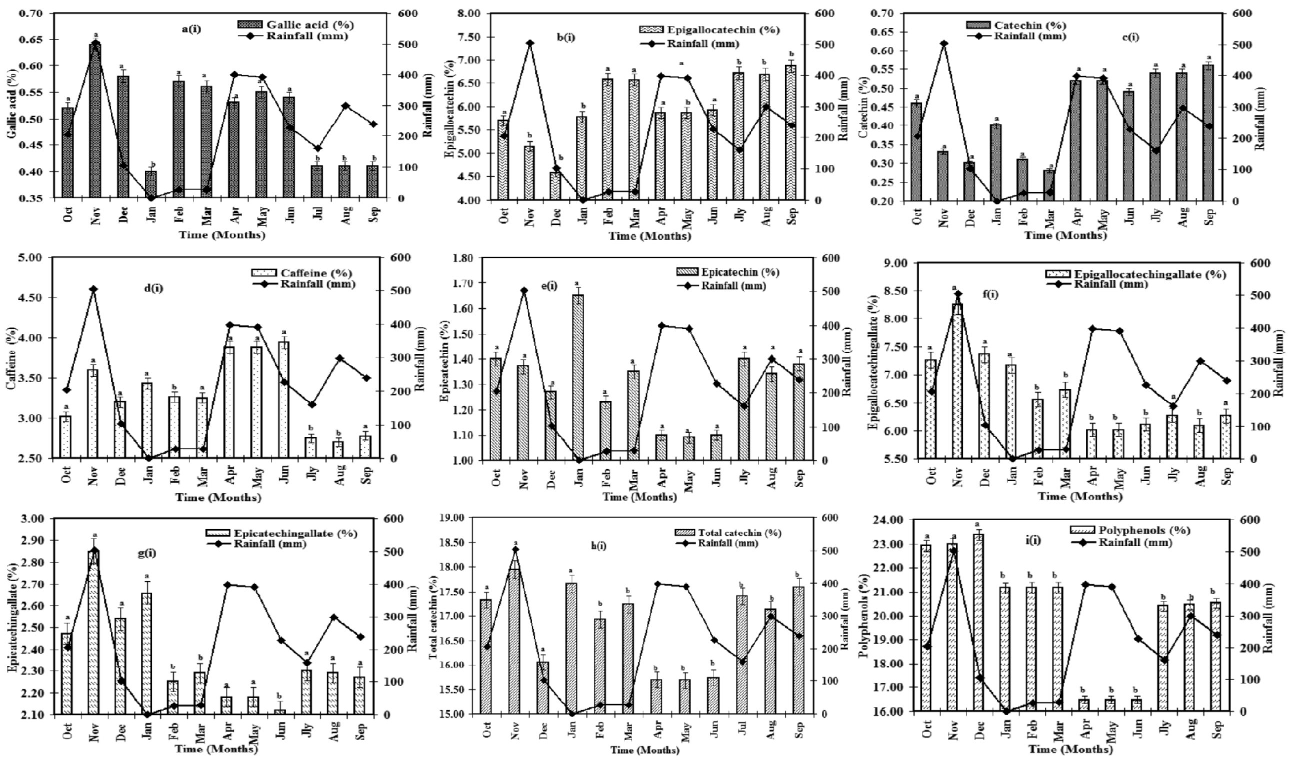

Fig. 3: Variations in polyphenols and catechin profile of non-aerated green tree tea processed from seedling tea cultivated with varying rainfall patterns in 2011-2012.

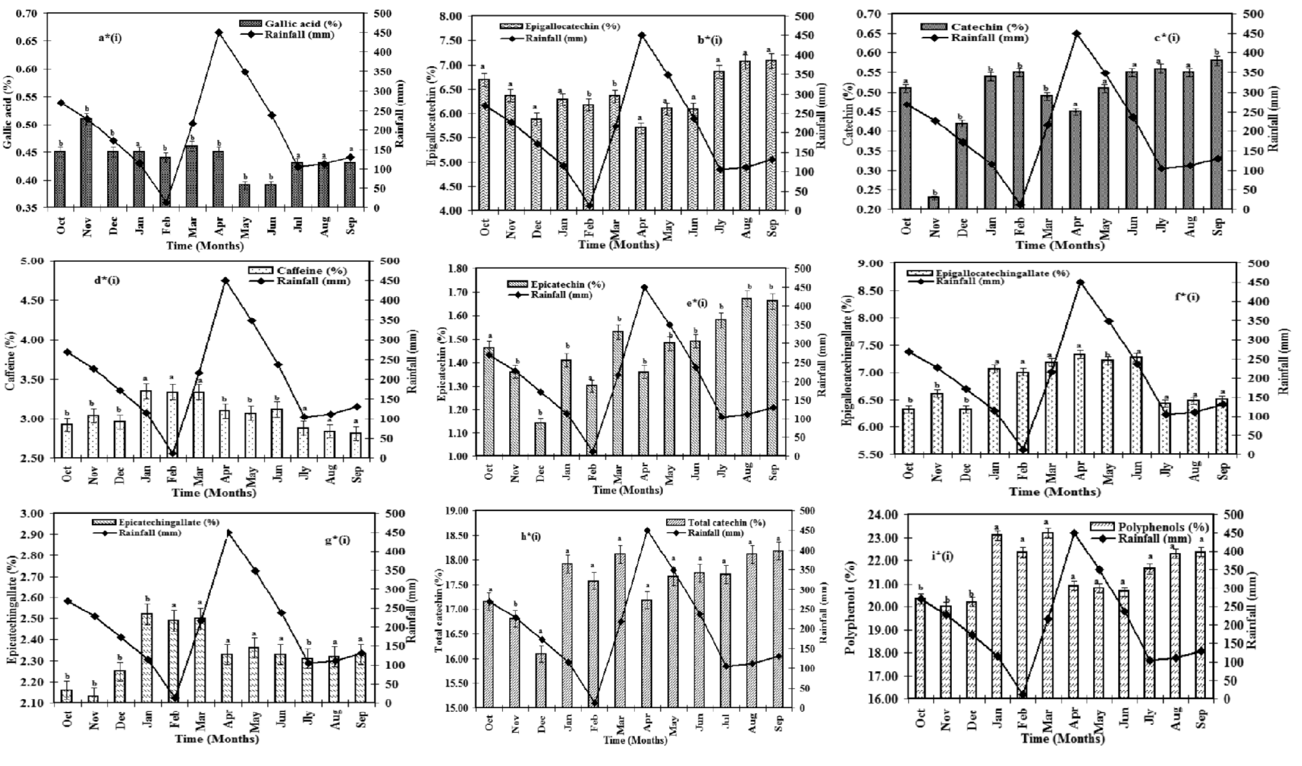

Fig. 4: Changes in monthly polyphenols and catechin profiles of non-aerated green tea seedling tea with varying rainfall patterns in 2012-2013.

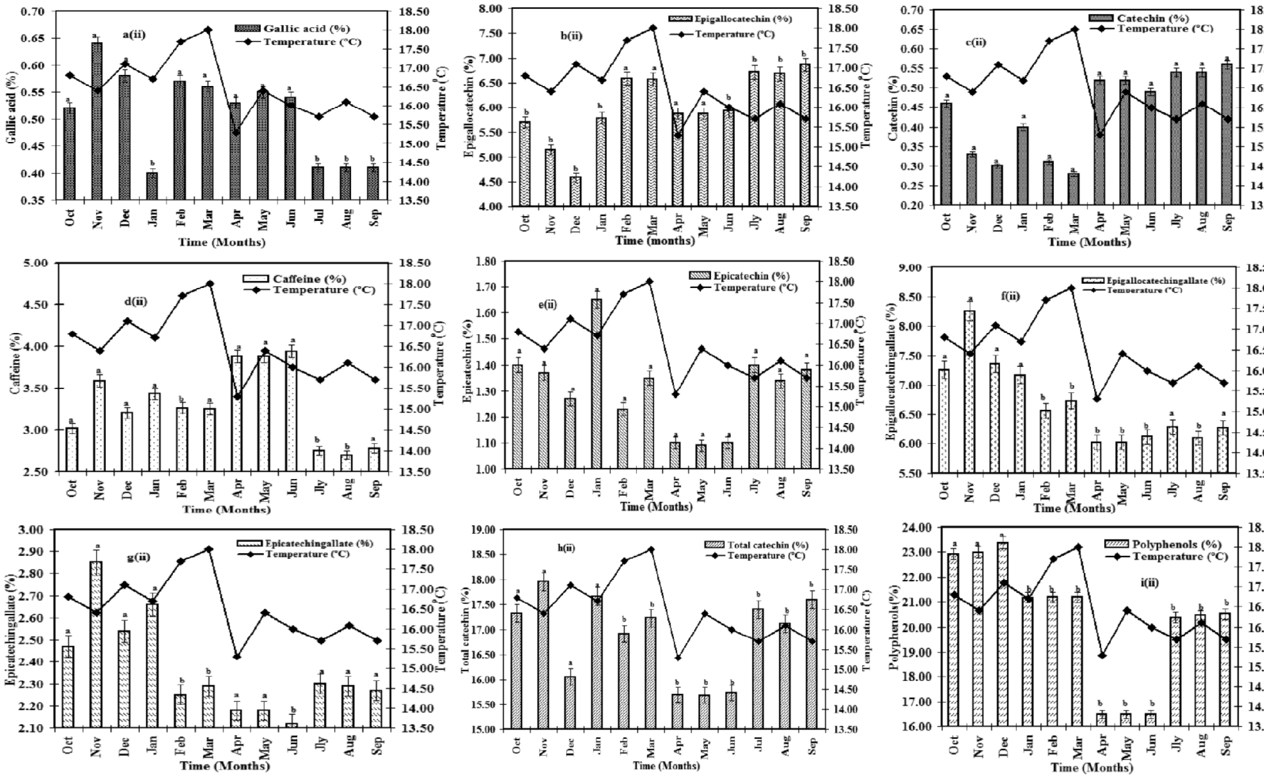

Fig. 5: The changes in polyphenols and catechin profiles of non-aerated green tea processed from seedling tea cultivated with varying mean monthly temperatures in 2011-2012.The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates. When absent the S.D value is p≤0.05 and falls within the dimensions of the points on the same curve. Means with similar letter(s) are not significantly different (p≤0.05).

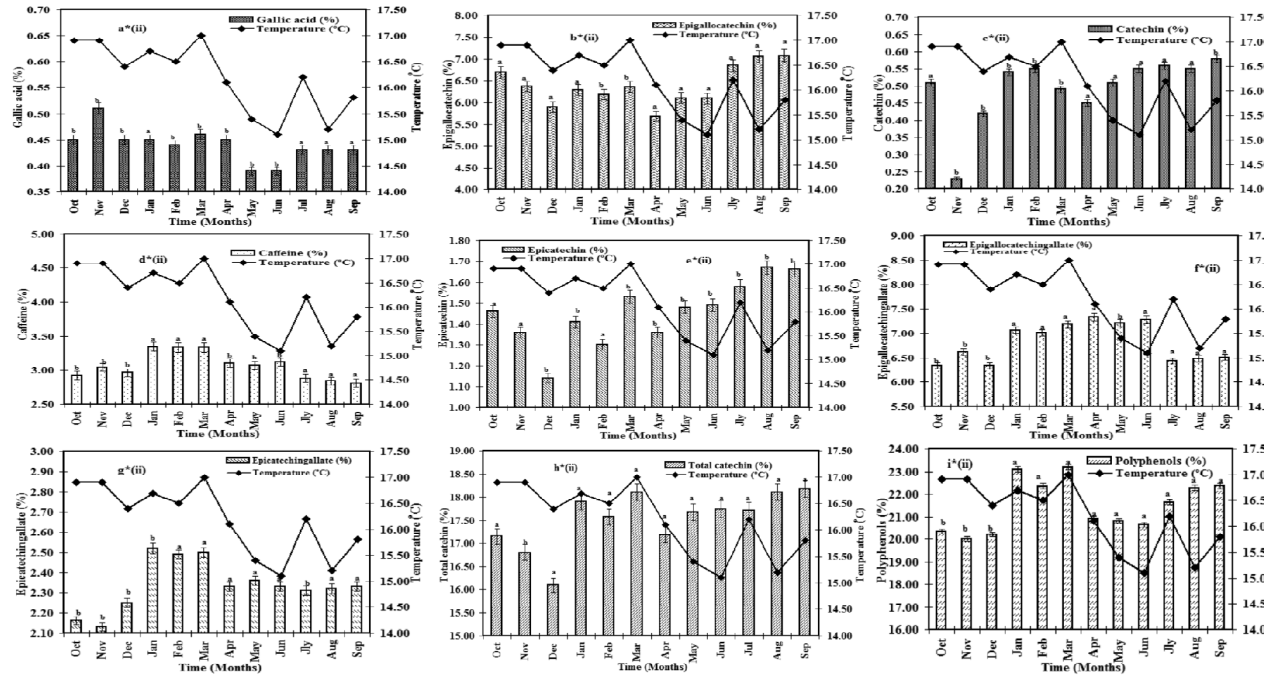

Fig. 6: The variations in catechin profiles of non-aerated green tea processed from seedling tea cultivated with varying temperature patterns in 2012-2013.

Table 3: Summary of rainfall and temperature recordings from October 2011-September 2013

Month	Rainfall (mm)	Temperature (˚C)	Rainfall (mm)	Temperature (˚C)	Rainfall (mm)	Temperature (˚C)
Jan			0	16.7	114.9	16.7
Feb			26.8	17.7	12.4	16.5
Mar			27.7	18.0	217.0	17.0
Apr			398.4	15.3	449.9	16.1
May			391.1	16.4	349.0	15.4
June			226.9	16.0	237.2	15.1
July			160.9	15.7	105.1	16.2
Aug			298.9	16.1	111.9	15.2
Sept			239.1	15.7	130.6	15.8
Oct	204.8	16.8	269.4	16.9		
Nov	503.7	16.4	227.6	16.9		
Dec	103.3	17.1	172.3	16.4		

The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates. When absent the S.D value is $p \leq 0.05$ and falls within the dimensions of the points on the same curve. Means with similar letter(s) are not significantly different ($p \leq 0.05$).

The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates. When absent the S.D value is $p \leq 0.05$ and falls within the dimensions of the points on the same curve. Means with similar letter(s) are not significantly different ($p \leq 0.05$).

The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates. When absent the S.D value is pd" 0.05 and falls within the dimensions of the points on the same curve. Means with similar letter(s) are not significantly different ($p \leq 0.05$).

The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates. When absent the S.D value is $p \leq 0.05$ and falls within the dimensions of the points on the same curve. Means with similar letter(s) are not significantly different ($p \leq 0.05$).

The vertical bars represent Standard deviation (S.D) of the mean of 3 replicates. When absent the S.D value is $p \leq 0.05$ and falls within the dimensions of the points on the same curve. Means with similar letter(s) are not significantly different ($p \leq 0.05$).

These low variations are due to the marginal changes in temperatures along the equator where these teas were produced. The observed changes could have resulted from the varying seasonal rainfall (Figures 3 and 4) and temperature patterns (Figures 5 and 6). Rainfall has a profound effect in nutrient absorption from the soil and is responsible for the variations in tea plant growth (*41*) and polyphenols (*21*) and other quality parameters (*36*) in different months in Kenya. These results reaffirm earlier observations that even with low changes in monthly and seasonal temperatures (*30*), the variations cause differences in tea quality. Overall, the changes in the quantities of tea polyphenols within the different months were related to the decrease in rainfall (Figures 3 and 4) and temperature (Figures 5 and 6) fluctuations. Similar observations had been recorded in past studies (*15, 20, 21*). The production of teas rich in high catechin and polyphenol compounds requires months that are warm and wet or cold and wet according to these research findings.

In conclusion, increasing nitrogen fertilizer application rates up to 400 kg N/ha/year improves quantities of catechins and total polyphenols in seedling non-aerated green tea. The production of low caffeine content of non-aerated green teas requires the application of low rates of nitrogen fertilizers or no application at all. Rainfall and temperature fluctuations caused variations in the non-aerated green tea quality parameters. However, the variations were much lower than those observed further away from the equator. The seasonal quality variations of non-aerated green tea from Kenya are therefore low.

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