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Effect of different levels of nitrogen on the chemical composition of tea (*C. sinensis* L) grown at higher altitude

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Abstract
The effect of different levels of nitrogen on water extracted substances (WE), total polyphenols (TP), epigallocatechin (EGC), epicatechin (EC), caffeine and amino acids in fresh tea leaves grown at higher altitude (1500 meter from sea level) and plucked in various months of the years, was studied. After plucking the tender leaves (bud+3) from tea bushes, the leaves were oven‘s dried at 60 ^{o}C for 24 hours. The dried leaves were used for determination of water-extracted substances, total polyphenols and amino acid contents by using 751G UV-spectrophotometer and EGC, EC and caffeine contents were determined by the high performance liquid chromatography (HPLC). The nitrogen level did not affect the WE significantly. The data show that TP content was increased up to certain level with the increase of nitrogen level (180 kg ha) but decreased with further increase of nitrogen. From treatment T4 (N=180, P=30, K=90 kg ha-1).TP increased significantly by the application of 180 kg N ha-1. The EGC, EC contents increased up to certain level with the increase of nitrogen and then declined after the application of 180 kg ha-1. The nitrogen level did not affect the caffeine contents.

Key Words: *C. sinensis* L, polyphenols, amino acid, EGC, caffeine, altitude, Pakistan.

Introduction
Fertilizer application is one of the major agro-inputs contributing to the cost of production and productivity in tea plantation. Tea is normally grown as a long term monoculture. For proper maintenance of the health of tea bushes and to obtained high yield, a well-balanced fertilizer application and manuring is necessary at certain intervals throughout the year. Amongst the various essential elements for tea becoming a leaf crop, nitrogen probably is the key element in plant nutrition. It accounts for about 4-5% of the dry weight of the shoot plucked for processing tea. Nitrogen is essential for growth and development of all living tissues, promotes branching, leaf production, enlargement of leaf surface and yield. Between 40-50% of the protoplasm is made up of nitrogenous compounds and 18-20% of dry protein is in the form of nitrogen **[1]**. Tea crop consists of leaves; the plant is therefore sensitive to application of nitrogen fertilizer. Quality of processed tea broadly depends on the composition of leaves, which in turn is influenced by nutrition. The optimum level of nitrogen is best derived from the quadratic relationship that exists between nitrogen levels and yield. Any increase above the optimum caused reduction in dry matter contents and increase in fiber contents, which affected the quality of the manufactured product in Malawi **[2]**. The China variety in particular and the hybrid in general produce very fine, flavor tea when they are grown above an altitude of about 1100 meal. The brightness, briskness and strength of the liquor assess quality of tea. Aroma and flavor add more to the overall quality. Formation and biogenetic pathway of chemicals responsible for quality are weather dependent and even a small change in the climatic factors can result in the significant change in the composition of the chemicals in the plucked shoot. Hilton **[3]** studied the concentrations of epigallocatechin, epigallocatechin gallate, gallocatechin and epicatechin gallate in the southern hemisphere. These catechins exhibit prime importance in specifying the quality. Hilton *et al.* **[4]** described that the concentration of flavanols in fresh apical shoots of tea was highest during the cold season.

Shoots plucked during the slow growth conditions contained a higher proportion of catechin and epigallocatechin (EGC) being most significantly affected. Goswani and Barbora **[5]** mentioned that quality of made tea is dependent on the caffeine content and flavanol glycosides of fresh leaves. Cheng **[6]** studied the effect of season and climatic factors on the shoot growth and chemical composition of tea tree and stated that at higher altitudes total polyphenol, caffeine and catechin contents are increased and positively correlated with relative humidity. The apical shoots of tea consisting ideally of the terminal bud and the adjacent leaves known as 'tea flush' make up the raw material, which is processed to make black, green or other forms of tea. The flush contains the group of polyphenolic compounds, which are responsible for the unique character of tea. These compounds are mainly flavanols, together with flavonols and flavonol glycosides, flavones, acids and depsides. During the processing of black tea, about 90-95% of the flavanols undergo enzymatic oxidation to products which are directly responsible for the characteristic colour of tea brews, their astringency and unique taste. The present study was designed at 1500 mael to determine the chemical composition of fresh tea leaves with varying levels of nitrogen at higher altitude of potential tea growing area of Pakistan.

2. Materials and Methods

The research study was conducted on seven years old tea bushes (Chinese Qi-Men) at village Battal (1500 masl), district Mansehra during the year 2010, 2011 and 2012. The Physico-chemical characteristic of experimental site is given in **Table 1**. The study was organized under the Randomized Complete Block Design with eight treatments replicated four times. The treatments were kept T_1 = control, T_2 = 0-30-90, T_3 = 120-30-90, T_4 =180-30-90, T_5 =240-30-90, T_6= 300-30-90, T_7 =360-30-90, T_8 420-30-90 kg N-P-K ha^{-1} an^{-1} in three split doses. The sources for nutrients application were ammonium sulphate, di-ammonium phosphate, triple super phosphate and potassium sulphate. Other agronomic practices were applied uniformly to all the treatments. The tender shoots "bud+3" were plucked from the tea bushes of each treatment and placed in the oven at 60 ^{0}C for drying for 24 hours to avoid fermentation process before the analysis of other chemical components of the leaves. The dried leaves were ground in the grinder and placed in the desiccator. Liquor was prepared and samples were analyzed for moisture contents, water extracted substances (WE,) total polyphenols (TP), caffeine and amino acid contents, by using 751G UV-Spectrophotometer, as described by Anonymous [7] High-tech Agriculture Co., Ltd. China. For the determination of epigallocatechin (CGC) and epicatechin (EC), the High Performance Liquid Chromatography (HPLC) system was used to obtain the chromatograms of fresh tea leaves "bud +3. The samples of the tea leaves were prepared for analysis by using aqueous extraction procedure, which simulated actual brewing conditions for a cup of tea [8].

2.1 Statistical analysis

The data were collected for consecutive three years and statistically analyzed by using one-way analysis of variance and multiple range test. Analyses were carried out by using the statistical soft were Minitab [9] and Statgraphics [10].

3. Results and discussion

3.1. Moisture contents

The moisture content of the tea leaves, plucked in the various months of the year and dried in the oven at 60^o C, is given in Tables 2-6. The highest percentage of moisture content in the tea leaves was obtained in treatment T_6 (9.96$\pm$1.92%) and the lowest in treatment T_5 (7.00$\pm$0.72%) during the month of May (Table-2).The mean value for the moisture content was 8.59$\pm$1.74% for all the 8 treatments from the harvest during the month of May. Subsequently, in the month of June the highest percentage of moisture content was obtained (9.28%) form the plants given the treatment T_6 and lowest in the plants receiving treatment T_7 (7.85%). The average percentage of moisture content for all the eight treatments was 8.62$\pm$0.56% (Table-3). From the plants harvested in the month of July, the moisture content ranged from 7.95$\pm$0.61% in treatment T_8 to 8.65$\pm$0.53% in treatment T_1. The mean value of all the eight treatments was 8.30 $\pm$ 0.55% for the month of July (Table-4). In the month of August the highest percentage of moisture content was 8.78$\pm$0.63% in plants treated with treatment T_2 and lowest was 6.81$\pm$0.81% in treatment T_8. The average percent for all the eight treatments was 7.65$\pm$1.06% (Table-5). The last month of plucking season was September in which the highest

% of moisture content (9.01±0.63%) was present in treatment T_3 and lowest (6.96±0.66%) in was in treatment T_6. The average value of moisture content was 7.87±0.89% (Table-6) Though the moisture content of tea leaves were different for the different levels of nitrogen treatment and for different plucking months, at higher altitudes (1500 masl), but the difference was statistically not significant. It seems that variation in the altitude and fertilizer doses may not be playing a significant role in moisture content of the fresh tea leaves.

Table 1: Physico-chemical characteristics of the experimental plot at Battal (pre-experiment)

Properties	Unit	Soil depth		
		15 cm	30 cm	45 cm
Sand	%	51	47	43
Silt	%	29	29	31
Clay	%	20	24	26
Textural Class	-	Loam	Loam	Loam
ECe	mS kg^{-1}	0.34	0.26	0.20
Soil Ph	-	5.71	5.91	6.37
Organic matter	%	1.28	1.10	0.97
Available K	mg kg^{-1}	48	52	52
PO_4-P	mg kg^{-1}	13.35	10.57	5.63
NO_3-N	mg kg^{-1}	1.27	0.98	0.70
Zn	mg kg^{-1}	4.6	4.8	3.4
Cu	mg kg^{-1}	4.8	4.6	2.6

3.2. Water extracted substances

The highest percentage of water-extracted substances in the tea leaves was in treatment T_3 35.29±11.4%) and the lowest in treatment T_7 (18.09±7.69%) during the month of May (Table-2). The mean value for the water-extracted substances was 25.13±8.29% for all the 8 treatments from the harvest during the month of May.

Table 2: Effect of different levels of nitrogen on the selected chemicals of fresh tea leaves in the month of May [1,2]

Treatments	Moisture	Water Extracted Substance	Poly Phenols	EGC	EC	Caffeine	Amino Acid
T_1	9.06±1.06	29.03±1.69	$11.33±0.01^{a}$	1.72±0.03	0.88±0.13	3.68±0.53	$1.81±0.05^{a}$
T_2	9.52±2.62	28.57±1.31	$12.19±0.41^{ab}$	2.08±0.06	0.86±0.06	4.29±0.40	$1.80±0.07^{a}$
T_3	9.58±1.51	35.29±11.40	$13.82±2.15^{bc}$	1.92±0.03	0.90±0.08	3.93±0.28	$1.86±0.06^{a}$
T_4	8.83±1.48	27.99±2.59	$16.64±1.53^{d}$	2.11±0.01	1.18±0.03	4.32±0.01	$1.85±0.07^{a}$
T_5	7.00±0.72	22.56±4.47	$14.72±0.80^{cd}$	2.45±0.28	1.16±0.22	4.23±0.30	$1.84±0.06^{a}$
T_6	9.96±1.92	19.86±8.60	$15.03±0.22^{cd}$	2.20±0.17	0.97±0.15	4.40±0.22	$1.93±0.10^{a}$
T_7	7.35±1.03	18.09±7.69	$13.53±0.33^{bc}$	2.03±0.10	0.79±0.32	4.49±0.20	$1.91±0.07^{a}$
T_8	7.45±1.77	19.69±10.89	$14.28±0.80^{bc}$	1.91±0.01	0.79±0.20	4.53±0.24	$1.93±0.07^{as}$
Mean	8.59±1.74	25.13±8.29	13.94±1.80	2.07±0.20	0.94±0.20	4.24±0.36	1.87±0.08

[1] M± S.D.; n =3 (May 2010 - 2012)
[2] Mean followed by different superscripts in the columns are significantly different at P< 0.001

Subsequently, in the month of June the highest percentage of water extracted substances was obtained (30.52%) form the plants given the treatment T_2 and lowest in the plants receiving treatment T_7 (21.61%). The average percentage of water-extracted substances for all the eight treatments was 26.66±3.48% (Table-3). From the plants harvested in the month of July, the water extracted substances ranged from 24.83±4.44% in treatment T_7 to 29.62 ±1.17% in treatment T_5. The mean value of all the eight treatments was 27.17 ± 7.42 % for the

month of July (Table-4). In the month of August the highest percentage of water extracted substances was 36.34±2.26% in plants treated with treatment T_2 and lowest was 26.84±2.26% in treatment T_7. The average percent for all the eight treatments was 30.63±3.99% (Table-5). The last month of plucking season was September in which the highest % for water-extracted substances (31.86±2.89%) was present in treatment T_6 and lowest (24.86±8.11%) in treatment T_1. The average value of water extracted substances was 28.27±5.02% (Table-6) It was evident from the present findings that the water extracted substances had no significant difference with the change in fertilizer doses at experimental site at higher altitudes. It means that the variation in the altitude and fertilizer doses did not play a significant role. Genetic variation may be of more importance for these components as indicated by these results.

3.3. Polyphenol contents of tea leaves

Young green tea shoots are extremely rich in polyphenolic compounds, the largest group being the catechins, which constitute up to 30% of the dry weight of material. Although there are other classes of the phenols present in green tea shoots, all are present at relatively low concentrations. Other plant species synthesize flavanols compound, but Camellia sinensis is unique in the range it contains the flavanols [11]. The quantities and proportions of polyphenols present in fresh tea leaves generally, are reflected in the chemical composition of made tea in the liquor characters. It is, therefore, expected that as the level of total polyphenol in fresh tea leaves rises, the quality of black tea will also improve and that would translate into better taster's evaluation [12]. During the present study the polyphenols content of the fresh tea leaves were determined during the different months of the plucking seasons.

Table 3: Effect of different levels of nitrogen on the selected chemicals of fresh tea leaves in the month of June. [1,]

Treatments	Moisture	Water Extracted Substance	Poly Phenols	EGC	EC	Caffeine	Amino Acid
T_1	8.84	28.99	11.55	1.89	0.66	3.87	1.37
T_2	9.04	30.52	13.05	1.94	0.63	3.68	1.35
T_3	9.11	30.23	14.28	1.85	0.69	3.73	1.42
T_4	8.79	28.81	16.19	1.91	0.84	3.76	1.39
T_5	7.98	25.98	15.04	2.01	0.56	3.80	1.41
T_6	9.28	24.43	14.30	1.88	0.55	3.95	1.45
T_7	7.85	21.61	13.70	1.82	0.60	3.94	1.45
T_8	8.08	22.67	14.30	1.74	0.52	3.97	1.46
Mean	8.62±0.56	26.66±3.48	14.05±1.37	1.85±0.11	0.63±0.10	3.84±0.11	1.41±0.04

[1] n =1

The polyphenol contents in fresh leaves plucked from various treatments supplemented with different levels of nitrogen is presented in Tables 2-6. Data presented in Table- reveals that the highest polyphenols content was obtained during month of May in treatment T_4 i.e. 16.64±1.53% and lowest in treatment T_1 (control) i.e. 11.3±0.01%. The mean value of all the 8 treatments was 13.84±1.80%. The polyphenol contents of tea leaves in treatment T_4 was significantly higher ($P<0.05$) as compared to treatment T_1 (controls) and treatments T_2, T_3, T_4 and T_8 and non significantly higher than treatment T_5 and treatment T_6. This observation suggests that nitrogen dose above 180 kg ha^{-1} is not beneficial for the quality of tea and could not help any more in improving the quality of tea.

During the month of June, the polyphenol contents ranged from 11.55% in treatment T_1 to 16.19% in treatment T_4. The average of all the 8 treatments for polyphenol contents during the month were 14.05±1.37% (Table-3). The higher doses than treatment T_4 show the decrease in polyphenol content. In the month of July, the polyphenol contents of tea leaves ranged from 11.78±0.42% for treatment T_1 to 15.08±0.43% for treatment T_3 with an average value of 13.62±1.23% for all the 8 treatments (Table-4). All the doses of nitrogen significantly increased the polyphenols contents in fresh tea leaves over control (T_1 and T_2) and were highest in treatment T_3, but declined for the remaining higher doses of nitrogen. In the month of August, the polyphenols content of tea leaves grown at higher altitude ranged from 11.98±0.33% for treatment T_1 to 15.31±0.33% for treatment T_4 with an average value of 13.45±1.31% for all the 8 treatments (Table-5). During the month of September, the polyphenols

contents ranged from 11.61+0.16% for treatment T_1 to 15.01±0.29% for treatment T_4 .The mean value for all the 8 treatments was 13.34±1.23%. The polyphenols content increased with the nitrogen level up to treatment T_4 but decline beyond this level (Table-6).

Table 4: Effect of different levels of nitrogen on the selected chemicals of fresh tea leaves in the month of July. [1,2]

Treatments	Moisture	Water Extracted Substance	Poly Phenols	EGC	EC	Caffeine	Amino Acid
T_1	8.65±0.53	23.43±11.83	11.78±0.42^{a}	1.56±0.38	0.60±0.03	3.51±0.46	1.44±0.11
T_2	8.37±0.59	29.60±9.98	13.29±0.59^{ab}	1.70±0.25	0.59±0.11	3.89±0.24	1.13±0.12
T_3	8.27±0.42	26.83±12.38	15.08±0.43^{bc}	1.60±0.35	0.64±0.06	3.68±0.25	1.51±0.11
T_4	8.44±0.28	24.95±10.42	14.85±0.80^{bc}	1.92±0.04	0.79±0.06	3.83±0.05	1.56±0.18
T_5	8.26±0.59	29.62±1.17	13.51±2.06^{bc}	1.91±0.13	0.88±0.00	3.92±0.08	1.62±0.24
T_6	8.16±0.68	31.16±2.06	13.38±0.63^{bc}	1.86±0.02	0.65±0.13	4.01±0.16	1.63±0.25
T_7	8.33±1.00	24.83±4.44	13.52±0.32^{bc}	1.75±0.03	0.68±0.06	4.10±0.22	1.63±0.24
T_8	7.95±0.61	27.78±1.97	13.58±0.62^{bc}	1.69±0.21	0.61±0.10	4.11±0.25	1.62±0.25
Mean	8.30±0.55	27.17±7.42	13.62±1.23	1.75±0.21	0.68±0.11	3.88±0.27	1.56±0.19

[1] M± S.D.; n =3 (July 1998 to 2000)

[2] Mean followed by different superscripts in the columns are significantly different at P< 0.001

The polyphenol contents significantly increased in treatment T_4 from treatment T_1. The data show that the polyphenols content were increased up to certain level with the increase of nitrogen level (T_4) but decreased with further increase of nitrogen from treatment T_4 (N=180, P=30, K=90 kg ha^{-1}). Willson and Choudhury [13] observed the reduction in quality with the increase in nitrogen in Kenya. Nakagawa *et al.* [14] pointed out that the shoot of the first crop had the greater content of amino acids and lower content of flavanols than the second and third crops plucked in summer. He indicated the range of flavanols during the month of May from 13.0-14.0 % whereas in June from 15.2-16.2%. According to Takayanagi **[15]** the flavanols during the month of April was 9.2- 11.7% and during May it was 9.1-9.7%. The findings are comparable with the results of the present study.

3.4. Epigallocatechin (EGC)

The EGC contents of the tea leaves plucked from various treatments with different levels of nitrogen are presented in Table 2-6. The EGC content during the month of May ranged from 1.72±0.03% in bushes given the treatment T_1 to 2.45±0.28% in treatment T_5 with the average values of (2.07±0.20%) for all the 8 treatments. The values in different treatments show the variation but they were statistically non significant (Table-2) During the month of June, the highest quantity of EGC was obtained from the bushes grown in treatment T_5 i.e. 2.01% and the lowest quantity form bushes given the treatment T_8 i.e.1.74% (Table-3). The mean value for all the 8 treatments was 1.85±0.11%. The EGC contents in tea bushes harvested in the month of July ranged from 1.56±0.38% in treatment T_1 to 1.92±0.04% in treatment T_4 (Table-4). The average value for all the 8 treatments was recorded as 1.75±0.21%. The doses of nitrogen up to 180 kg ha^{-1} nitrogen in treatment T_4 increased the EGC contents and declined for the remaining higher doses of nitrogen (T_5-T_8). Though the values show the variation for different treatments but were statistically non significant. Similarly, the Table-5 reveals that the EGC contents were at peak in bushes grown in the presence of treatment T_5 (1.74±0.11%) during the month of August. The lowest amount was observed in treatment T_1 (1.47±0.40%). The average value for all the 8 treatments was 1.63±0.22%. All the treatments with high doses of nitrogen show an increase in EGC as compared to treatment T_1 (control) in which fertilizer was added but there was decline for the higher doses (T_6-T_8). The EGC contents in fresh tea leaves was the highest in T_5 (N=240, P=30, K= 90 kg ha^{-1}) during the months of May, June and August during the experimental years. During July the treatment T_4 (N=180, P=30, K=90 kgha^{-1}) gave the highest contents of EGC at 1500masl (Tables 2-6).

Table 5: Effect of different levels of nitrogen on the selected chemicals of fresh tea leaves in the month of

August. [1,2]

Treatments	Moisture	Water Extracted Substance	Poly Phenols	EGC	EC	Caffeine	Amino Acid
T_1	7.66±0.33	28.96±0.58	11.98±0.33^{a}	1.49±0.40	0.56±0.03	3.45±0.54	1.33±0.04
T_2	8.78±0.63	31.10±1.04	12.16±0.80^{ab}	1.58±0.31	0.61±0.03	3.80±0.06	1.33±0.02
T_3	8.77±1.10	36.34±2.31	14.09±1.49^{ab}	1.53±0.37	0.56±0.06	3.47±0.52	1.42±0.05
T_4	7.35±0.25	32.95±5.67	15.31±1.10^{bc}	1.64±0.35	0.69±0.03	3.73±0.20	1.37±0.03
T_5	7.05±0.45	29.14±1.02	14.31±0.35^{bc}	1.74±0.11	0.69±0.08	3.68±0.00	1.41±0.04
T_6	6.82±0.68	32.49±4.83	12.42±0.43^{ab}	1.73±0.16	0.62±0.07	3.84±0.16	1.45±0.05
T_7	7.94±1.81	26.84±2.26	13.14±0.10^{ab}	1.67±0.13	0.55±0.03	3.89±0.12	1.44±0.05
T_8	6.81±0.81	27.21±2.32	14.17±0.40^{ab}	1.63±0.14	0.52±0.01	3.96±0.12	1.44±0.03
Mean	7.65±1.06	30.63±3.99	13.45±1.31	1.63±0.22	0.60±0.07	3.73±0.28	1.40±0.06

[1] M± S.D.; n =3 (August 2010 to 2012)
[2] Mean followed by different superscripts in the columns are significantly different at P< 0.001

3.5. Epicatechin (EC)

The epicatechin contents of the tea leaves plucked in different months and supplemented with different levels of nitrogen are shown in the Tables 2-6. The data in Table 2 and 3 show that during the months of May and June, the highest epicatechin contents of the tea leaves were present in plants given the treatment T_4 i.e. 1.18±0.03% and 0.84% and the lowest in treatment T_8 (N=420, P=30, K=90 kg ha^{-1}) i.e. 0.79±0.20% and 0.52% respectively. The mean values of EC from the plant harvested in the months of May and June recorded for all the 8 treatments were 0.94±0.20% and 0.63±0.10% respectively. The EC contents increased with the increase of nitrogen level up to treatment T_4 but declined for treatments T_6 to T_8 when the higher doses of nitrogen were applied during May (Table-2). In the month of July the EC ranged from 0.59±0.11% in treatment T_2 (N=240, P=30, K=90 kg ha^{-1}) (Table-4). During the month of August the bushes grown in presence of treatment T_5 gave the highest EC content of 0.69±0.08% while treatment T_8 (N=420, P=30, K=90 kg ha^{-1}) shows the lowest EC contents (Table-5). The mean value of all the 8 treatments was observed to be 0.68±0.11% in July and 0.60±0.07% in the month of August. During the present findings, EGC and EC quantity was high in the first flush. It may be due to the preceding dormant winter season, the quantity of EGC and EC was found steady during the regular plucking season from May to September at both the experimental sites. These results are also in line with the results of Cloughley **[16]**.

Table 6: Effect of different levels of nitrogen on the selected chemicals of fresh tea leaves at in the month of September. [1,2]

Treatments	Moisture	Water Extracted Substance	Poly Phenols	Amino Acid
T_1	8.04±0.22	24.86± 8.11	11.61±0.16a	1.62±0.03
T_2	7.65±0.36	29.60±7.55	11.83±0.30a	1.61±0.05
T_3	9.01±1.68	30.00±9.07	14.48±0.57^{ab}	1.70±0.09
T_4	7.68±0.25	26.45±7.62	15.01±0.29^{ab}	1.66±0.06
T_5	7.22±0.46	29.60±1.00	13.16±0.75^{ab}	1.64±0.01
T_6	6.96±0.66	31.86±2.89	13.14±1.15^{ab}	1.70±0.06
T_7	8.33±1.37	25.76±4.73	13.79±0.83^{ab}	1.69±0.05
T_8	8.05±0.63	28.03±1.15	13.65±0.35^{ab}	1.72±0.07
Mean	7.87±0.89	28.27±5.02	13.34±1.23^{ab}	1.67±0.06

[1] M± S.D.; n =3 (September 2010- 2012)
[2] Mean followed by different superscripts in the columns are significantly different at P< 0.001

3.6. Caffeine

The effect of different levels of nitrogen on the caffeine contents in tea leaves is resented in Table 2-6. The caffeine contents of the tea leaves during the month of May ranged from 3.68±0.53% in treatment T_1 (control) to 4.53□0.24% in treatment T_8 (N=420, P=30,K=90 kg ha^{-1}) with a mean value of 4.24±0.36% for all the 8 treatments (Table-2). During the month of June, the highest contents of caffeine were in treatment T_8 (3.97%) and the lowest in T_2 (3.68 %) with the average value of 3.84±0.11% for all the 8 treatments (Table-3). Again, during the month of July and August the caffeine contents were at peak in treatment T_8 i.e. 4.11±0.25% and 3.96±0.12% respectively.

Table 7: Meteorological data of experimental site during study period

Months	2010				2011				2012			
	Temp. ^{0}C		RH %	Rainfall	Temp. ^{0}C		RH %	Rainfall	Temp. ^{0}C		RH %	Rainfall
	Min	Max		mm	Min	Max		mm	Min	Max		mm
Jan.	1.3	1.8	59.3	120	1.2	8.2	62.4	138	1.1	9.4	56.2	86.5
Feb.	1.9	10.8	77	70.91	3.9	10.6	63.4	109	-0.2	11.1	49.9	38.5
March	4.9	15.8	82.71	94.01	6	14.2	55.8	248	3.8	17.4	46.7	62.5
April	10	24.2	60	101.27	10.6	22.2	39.7	34	9.4	26	36.3	22.5
May	14.3	29.8	60.35	10.8	13.1	26.7	35.9	42	15.5	32.9	37.1	54.5
June	16.3	30.9	63.9	330	15.3	28.8	40.4	76	17.4	31.8	47.2	103.5
July	18.5	28.4	64	58.3	18.4	27.6	62.5	152	18.6	29.2	71.2	168
Aug.	17.4	25.2	77.5	105	17.2	25.3	71.1	1754	18	29.1	69.8	163
Sep.	14.9	21.8	82.7	92	15.3	25.8	67.6	160	16.1	27.3	63.4	198
Oct.	9	18.9	79	36	9.2	22.9	43.9	7	12.1	26.6	48.4	4
Nov.	4.1	12.7	60.5	0	6	14.8	52.7	11.8	5.2	18.3	47.8	4
Dec.	3.2	15.7	54.7	0	2.4	14.9	30.9	0	1.8	10.3	44.5	45.5
Mean	9.58	19.66	68.47	1018.29	9.88	20.1	52.19	2731.8	9.9	22.45	51.54	950.5

The control (T_1) shows the lowest range of caffeine contents in both the months of July and August i.e, 3.61±0.46% and 3.45±0.54% respectively (Table-4 &5). During the present investigation it was evident that the concentration of caffeine and its metabolism in the tea leaves is dependent on climatic factors, agronomic practices and altitude. The caffeine concentration was found in active growing season during the early flush. It remained steady during the regular plucking season and declined in the late plucking season. Similar observations were made by Teranishi and Hornstein **[17]** and Suzuki *et al.* **[18].**

3.7 Amino acids

The effect of different levels of nitrogen on tea leaves is presented in Tables 2-6. The data in Table-2 show that the amino acid contents during the month of May ranged from 1.80±0.08% in treatment T_2 (N=0, P=30, K=90 kg ha^{-1}) to 1.93±0.10% in treatment T6 (N=300,P=30,K=90 kg ha^{-1}). The average value for all the treatments was 1.87±0.08%. The amino acid contents showed the variation for different levels of nitrogen but the variation are statistically non significant. During the month of June, the highest amino acid contents were obtained in treatment T_8 i.e. 1.46% whereas; the treatment T_2 gave the lowest contents (1.35%) of amino acid (Table-3). The average value for all the 8 treatments was 1.41±0.04%. In the months of July and August the amino acid contents in tea leaves were at peak in treatment T_6 (N=300, P=30, K=90 kg ha^{-1}) i.e. 1.63±0.25% and 1.45±0.05% respectively. The treatment T_2 (N=0, P=30, K=90 kgha^{-1}) showed the lowest content of amino acids in both the months i.e. 1.13±0.12% in July and 1.33±0.02% in August (Table 4-5). The average value for all the 8 treatments during July was observed to be 1.56±0.19% and in August 1.40±0.06%. The amount of amino acid contents shows the variation but it was statistically non significant. During the month of September, the highest amount of amino acid contents was obtained in tea bushes, which were given treatment T_8 (1.72±0.07 %) against the treatment T_2 (1.61±0.05%) as lowest. The average for all the 8 treatments was 1.67±0.06%. Though the contents of amino acid show the variation in different treatments but statistically they were non significant (Table-6). The present study shows that the amino acids content increased with the increase of nitrogen. Takeo **[19]**

found in Japan that high rates of sulphate of ammonia $(NH_4)_2 SO_4$ improved the quality of green tea and increased the amino acids content of tea leaves.

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