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QUALITATIVE ASSESSMENT OF FRESH TEA PRODUCED IN PAKISTAN GROWING UNDER DIFFERENT AGROECOLOGICAL CONDITIONS AND FERTILIZER TREATMENTS

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Abstract

Pakistan is auspicious in having *ca.* 60,000 hectare of land in the north of the country with propitious ecological elements appropriate for tea cultivation. During the current investigation qualitative components of tea produced in Pakistan under different agroecological conditions were studied. To evaluate the effect of plucking season, altitude and agronomic practices upon quality of tea, trials were initiated in 1998 at Shinkiari (1000 m) and Battal (1500 m) on mature tea bushes. Nitrogen (N) treatments (120-420 kg/hec/annum) were applied to the experimental plots while potassium (K) and phosphorus (P) were kept constant in the range of 90 and 30 kg/hec/annum respectively. Tea leaf samples (two leaves and bud) were collected in four plucking seasons *i.e.* vernal, aestival, serotinal and autumnal in 1999. Epigallocatechin (EGC), Epicatechin (EPC) and caffeine were assayed using standard methods. The concentration of these constituents was found to be highest in the vernal samples at 180 kg/hec/annum of N treatment. Caffeine was found to be directly proportional to N fertilizer applied. Latitude was found to have pronounced effect on all tea leaf constituents. The crop plucked at 1500 m elevation had much higher concentration of these constituents than for the crop plucked at 1000 m elevation. Analysis of variance showed that increasing nitrogen treatments and different seasons had a significant influence ($p<0.05$) on EGC, EPC and caffeine level of tea leaves at both localities. The results show that tea produced in Pakistan contains constituents in premium concentration highly desirable for black tea processing.

Introduction

Tea is one of the most common beverages all over the world. Its popularity is attributed to its sensory properties, relatively low retail price, stimulating effects and potential health benefits. Tea plant *Camellia sinensis* L., originally from south–east China, gradually expanded to India, Sri Lanka and further into tropical and subtropical countries. Since the last decade of the 19th century tea is also produced in Pakistan, which has 60,000 hec of land in the northern highland suitable for tea cultivation (Rauf *et al.*, 1991; Rashid *et al.*, 1993). Successful tea cultivation in Pakistan and its economical viability in terms of yield has already been established (Hamid & Naseer, 1989; Rauf, 1991; Rauf *et al.*, 1991; Hamid *et al.*, 1993;).

To increase economic returns, tea growers tend to focus on increasing yields rather than improving the quality. The latter has received little attention due to the high consumer demands and lack of quality consciousness. However, attempts have been made to determine the quality of tea and factors affecting it (Owour *et al.*, 1990). The quality and chemical composition of the tea flush vary with climatic conditions (Howard, 1978; Cloughley, 1982; Goto *et al.*, 1996). Minor climatic variations are known to cause significant changes in the chemical composition and hence the quality of black tea (Owour *et al.*, 1987; Mahanta *et al.*, 1988). The frequency and magnitude of rainfall (Owour *et al.*, 1987a, b), high altitude (Owour *et al.*, 1990) and low temperature (Teranishi & Hornstein, 1995) are known to affect directly the plant characteristics. In addition, rate of application of nitrogenous fertilizer is known to cause changes in chemical composition and quality of tea (Hilton, 1973; Cloughley, 1983; Malenga, 1987; Owour *et al.*, 1987) and yield (Ranganathan & Natesan, 1987).

Various biochemical factors are known to influence tea quality, the most important ones are the flavanols or catechins due to their therapeutic values (Chen & Yu, 1994; Beecher & Merken, 2000) and a central nervous system stimulatory alkaloid called caffeine (Robertson, 1992; Teranishi & Horstein, 1995). Eight catechins have been identified till now, four of them constituting about one third of the dry weight of green tea (Beecher *et al.*, 1999) are of particular interest (Goto *et al.*, 1996).

Numerous attempts have been made to mention correlation between quality and the content of catechins (Hilton & Palmer-Jones, 1973; Ting, 1981; Obanda *et al.*, 1997), and caffeine (Cloughly, 1982; Owour *et al.*, 1990; Robertson, 1992; Teranishi & Horstein, 1995). These quality determining compounds are unanimously related with the surrounding climate and plucking seasons (Hilton *et al.*, 1973; Hilton, 1974; Cloughly, 1978-1979; Suzuki *et al.*, 1990; Goswani & Barbora, 1994; Cheng, 1995). It is accepted that high-grade tea is associated with high altitude, low temperature and slow growth (Owour *et al.*, 1987a; Owour *et al.*, 1990).

Cloughley (1982) and Ruan *et al.* (1997) and described increase in the caffeine content due to increased nitrogen fertilizer. Owour *et al.* (1987b) described that quality of tea deteriorates with increasing nitrogenous fertilizer rate due to diminishing synthesis of catechins (Willson & Choudhury, 1969; Hilton *et al.*, 1973). However, Rahman *et al.* (1978) reported that high levels of nitrogen increased the strength of the liquor. Malenga (1987) found that the quality of tea made from clones and hybrid seedlings was highest at a nitrogen application level of 100 kg/hec/annum. The present work describes the composition of fresh tea shoots grown at different altitude and the effect of different nitrogen concentrations on their chemical constituents.

Materials and Methods

Nitrogen treatments were given to tea plants grown at the National Tea Research Institute (NTRI) located in Pakhli Plain at 1000 m and Battal located in Konch Valley at 1500 m (Fig. 1). Eight treatments of NPK were applied to the experimental plots during the years 1998 and 1999, each of which had four replications. Treatment 1 (T_1) served as the control, which was not given any fertilizer treatment. In Treatment 2 (T_2) the ratio of NPK was kept 0:30:90 kg/hec/annum. The remaining Treatments (T_3-T_8) received varying proportions of N starting at a lower limit of 120 kg/hec/annum for T_3 and increasing by 60 kg/hec/annum to an upper limit of 420 kg/hec/annum for T_8.

Fresh, tender leaves that consist of two leaves and a bud were hand plucked during four seasons *i.e.* vernal, aestival, serotinal and autumnal (Odum, 1984) in 1999. The samples were preserved by drying for 2 hours in an oven at 95 ^{0}C and milled to a fine powder (Hilton *et al.*, 1973). Meteorological data from both the sites is presented in Table 1.

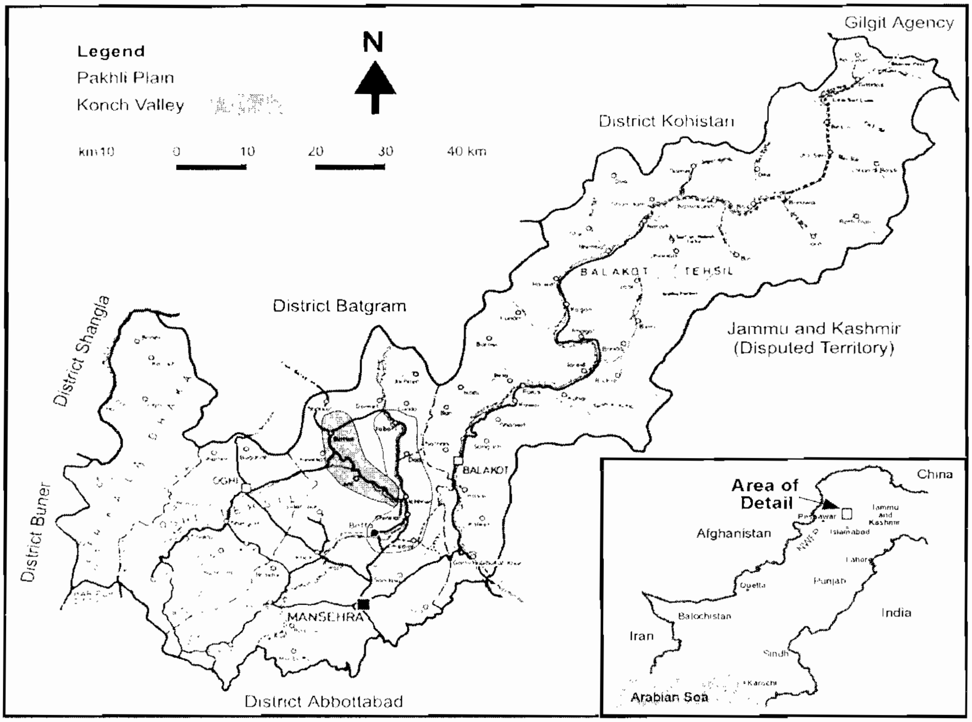

Fig. 1. Map of district Mansehra showing study locations.

Green tea extract was prepared using an aqueous extraction procedure (Dalluge *et al.*, 1998) and filtered twice. Analysis of epicatechin (EPC), epigallocatechin (EGC), and caffeine was performed at 280 nm using reversed phase HPLC (Shimadzu, Japan) system consisting of an assembly of two Shimadzu LC–GA pumps controlled by a Shimadzu SCL–6A automated gradient controller. A two-way, isocratic elution system was applied with a flow rate of 0.8 µl/minute. The solvent composition used was 60 % acetonitrile, diluted with de-ionised water. Nova Pak phenyle column (Waters Millipore Corporation) (60A°, 3.9×150 id) having 4 µm particle size was used for separation of catechins and caffeine (Goto *et al.*, 1996).

Results and Discussion

Data presented in Table 2 shows that in vernal season in treatment T_4 (NPK 180-30-90) EGC % of tea leaves was at the peak (1.84 %) at NTRI. Lowest quantity (1.02 %) was recorded during autumnal season in treatment T_3 (NPK 120-30-90), apart from the control. During the aestival and serotinal seasons, the content was inbetween the two extremes. The Fig. 2a shows the relationship between seasons and fertilizer treatments and their effect on EGC %. As nitrogen increased, EGC % also increased up to treatment T_4 (NPK 180-30-90), after which it declined. Among the seasons, EGC % decreased from vernal to serotinal season and a slight increase was observed in autumnal season.

Table 1. Monthly temperature, relative humidity and precipitation at NTRI and Battal during the year 1999.

Month	Location	Temperature °C		Relative Humidity %	Rainfall (mm)
		Min.	*Max.*		
January	NTRI	4.06	14.6	69	105.3
	Battal	1.2	8.2	62.0	138
February	NTRI	6.10	16.1	63	106.5
	Battal	3.9	10.6	63.4	109
March	NTRI	7.3	20.0	56	131.2
	Battal	6.0	14.2	55.8	248
April	NTRI	12.0	29.9	46	16.8
	Battal	10.6	22.2	39.7	34
May	NTRI	15.0	34.0	36	11.5
	Battal	13.1	26.7	35.9	42
June	NTRI	18.6	37.2	42	21.8
	Battal	15.3	28.8	40	76
July	NTRI	22.0	34.3	60	253.1
	Battal	18.4	27.6	62	152
August	NTRI	20.8	30.3	77	152.5
	Battal	17.2	25.3	71	175
September	NTRI	20.0	32.0	84	122
	Battal	15.3	25.8	67	160
October	NTRI	9.8	29.3	60	2.4
	Battal	9.2	22.9	43	7
November	NTRI	7.1	21.8	76	85.6
	Battal	6.2	14.8	52	118
December	NTRI	2.5	22.8	89	-
	Battal	2.4	14.9	30	-

Annual Rainfall at NTRI = 1008.7 mm, Annual Rainfall at Battal = 1259 mm

At Battal (Table 3) the highest quantity of EGC % was observed in treatment T_4 in vernal season (2.16 %). Treatment T_8 accumulated 1.27 %, the lowest quantity of EGC % in tea leaves in autumn season, other than the control (1.08 %). Figure 2b shows that as nitrogen fertilizer was increased there was an increase in the content of EGC % at Battal up to T_4, beyond which a decrease was observed. This trend was found to be the same in all treatments during all seasons. On comparing both the localities (Figs. 2a & b) samples from Battal presented a higher EGC % content in tea leaves over NTRI. Different treatments also showed a higher proportion of EGC % at Battal as compared to NTRI. Analysis of variance (Table 4) expressed that there was a significant effect ($p<0.05$) of seasons and treatments on EGC % in tea leaves at both the localities. Whereas, Seasons x Treatments interaction had a non-significant effect ($p<0.05$) on the EGC % of tea leaves.

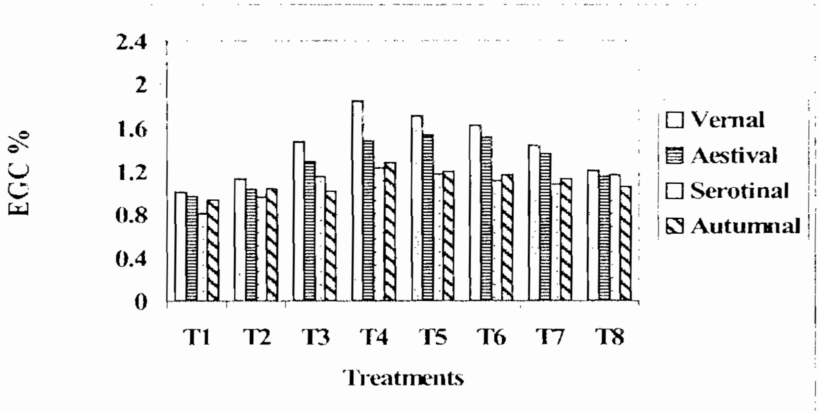

Fig. 2a. Variations in the EGC % in tea leaves at NTRI during different seasons under different N treatments at an altitude of 1000 m.

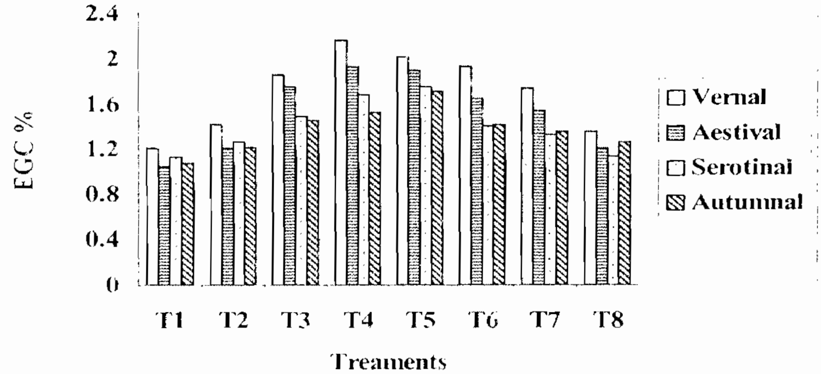

Fig. 2b. Variations in the EGC % in tea leaves at Battal during different seasons under different N treatments at an altitude of 1500 m.

At both the localities *i.e.* NTRI (Table 2) and Battal (Table 3) EPC % was highest during vernal season in treatment T_4. Comparing all the treatments, T_4 had a pronounced effect on the quantity of EPC % in all seasons, beyond which a decrease was observed at both the localities. Vernal season had higher content of EPC % at NTRI (Fig. 3a). While during the potential growing seasons, *i.e.* aestival and serotinal the proportion of EPC % was steady in T_3, T_5 and T_6 treatments. Figure 3b shows higher percentage of EPC at Battal than at NTRI. At both the experimental sites, initially there was increase in the EPC % up to T_4 in all the seasons, then it dropped gradually and at T_8 lowest EPC % of all the treatments was observed (Figs. 3a & b).

Analysis of variance (Table 4) shows a highly significant behaviour ($p<0.05$) of treatments and seasons on EPC % of tea leaves at both the localities. Interaction of EPC % of tea leaves among Seasons x Locality, Treatments x Locality, and Seasons x Treatments x Locality shows significant effects ($p<0.05$).

Table 2. Contents of three constituents (%) in fresh tea leaves collected from NTRI in different seasons under different N treatments.

Treatments	Vernal			Aestival			Serotinal			Autumnal		
	EGC %	EPC %	Caffeine %	EGC %	EPC %	Caffeine %	EGC %	EPC %	Caffeine %	EGC %	EPC %	Caffeine %
T_1	1.01	0.53	3.60	0.97	0.49	3.51	0.81	0.31	3.52	0.94	0.34	3.60
T_2	1.13	0.62	3.72	1.03	0.50	3.63	0.96	0.33	3.60	1.04	0.36	3.66
T_3	1.47	0.68	3.89	1.29	0.53	3.76	1.15	0.56	3.65	1.02	0.41	3.79
T_4	1.84	0.77	3.96	1.48	0.61	3.81	1.23	0.60	3.73	1.28	0.56	3.70
T_5	1.71	0.73	4.08	1.53	0.58	3.88	1.18	0.53	3.82	1.20	0.52	3.74
T_6	1.62	0.61	4.13	1.51	0.52	3.91	1.11	0.51	3.90	1.17	0.48	3.81
T_7	1.44	0.58	4.16	1.36	0.46	4.03	1.08	0.43	4.00	1.13	0.47	3.86
T_8	1.21	0.52	4.27	1.15	0.47	4.18	1.17	0.38	3.81	1.06	0.43	3.94

Table 3. Contents of three constituents (%) in fresh tea leaves collected from Battal in different seasons under different N treatments.

Treatments	Vernal			Aestival			Serotinal			Autumnal		
	EGC %	EPC %	Caffeine %	EGC %	EPC %	Caffeine %	EGC %	EPC %	Caffeine %	EGC %	EPC %	Caffeine %
T_1	1.21	0.67	3.75	1.05	0.50	3.61	1.13	0.30	3.63	1.08	0.32	3.51
T_2	1.42	0.81	3.83	1.21	0.58	3.68	1.27	0.36	3.66	1.22	0.34	3.56
T_3	1.86	0.86	4.01	1.75	0.68	3.74	1.49	0.61	3.71	1.46	0.59	3.68
T_4	2.16	0.97	4.27	1.93	0.73	3.77	1.68	0.68	3.79	1.53	0.63	3.76
T_5	2.01	0.83	4.13	1.89	0.66	3.84	1.75	0.59	3.86	1.71	0.60	3.81
T_6	1.93	0.64	4.20	1.65	0.54	3.96	1.41	0.51	3.92	1.42	0.56	3.90
T_7	1.74	0.52	4.26	1.54	0.47	4.11	1.33	0.46	3.98	1.36	0.51	3.92
T_8	1.36	0.41	4.35	1.21	0.39	3.86	1.14	0.35	3.85	1.27	0.42	3.96

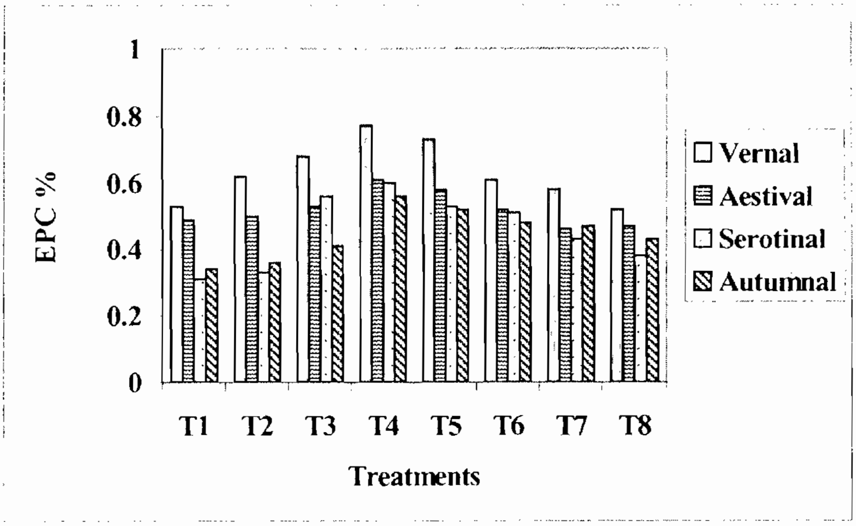

Fig. 3a. Variations in the EPC % in tea leaves at NTRI during different seasons under different N treatments at an altitude of 1000 m.

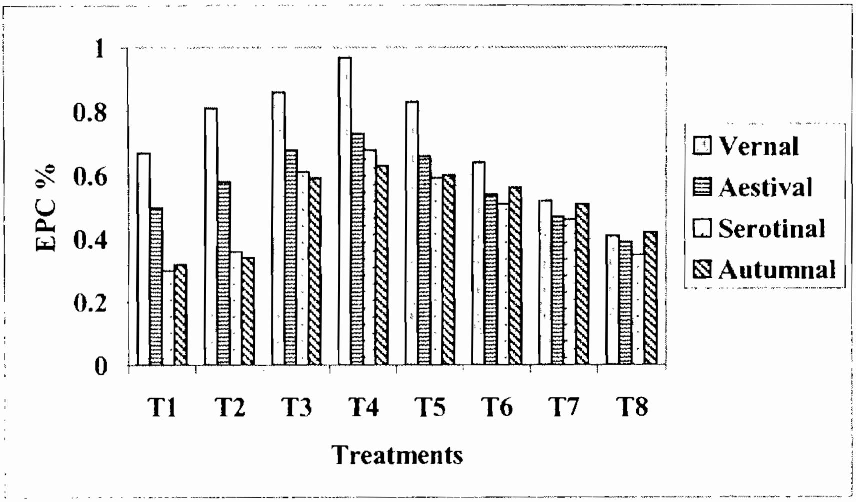

Fig. 3b. Variations in the EPC % in tea leaves at Battal during different seasons under different N treatments at an altitude of 1500 m.

Maximum caffeine value was observed in treatment T_8 (4.27 %) in the vernal season, whereas lowest value was found in control (3.51 %) in aestival season at NTRI (Table 3). The Fig. 4a shows that effect of nitrogen fertilizer is directly proportional to the caffeine content of the leaves at NTRI. Similar trend was also observed at Battal (Fig. 4b). There was no variation in the level of caffeine during aestival and serotinal seasons. In Battal (Table 3) maximum caffeine was found in treatment T_8 (4.35 %), in vernal season, whereas, minimum level for caffeine (3.51 %) was observed in autumnal harvest in control.

Table 4. Analysis of variance of EGC, EPC and Caffeine (%) in tea leaves collected from NTRI and Battal in different seasons under different N treatments.

Source	Degree of freedom	Sum of squares	Mean of squares	F-value	Significance
Replication	2	0.016	0.008	3.3209	n.s.
Seasons	3	1.302	0.434	182.964	**
Treatments	7	1.646	0.235	99.1197	**
Seas. x Treat.	21	0.422	0.020	8.4630	n.s.
Locality	1	0.131	0.131	55.1183	n.s.
Seas. x Locality	3	0.020	0.007	2.8426	**
Treat. x Locality	7	0.169	0.024	10.1931	**
Seas x Treat. X Locality	21	0.099	0.005	1.9828	**
Error	126	0.299	0.002		
Total	**191**	**4.103**			

**= Significance at 5 % probability, n.s.= Non-significant, Coefficient of variation = 9.03 %

Analysis of variance (Table 4) shows that increasing nitrogen treatments and different seasons had a significant influence ($p<0.05$) on the caffeine level of tea leaves whereas locality had no significant effect ($p<0.05$) on the caffeine content. Analysis of variance for interaction between Seasons x Treatments had no significant effect ($p<0.05$) on the caffeine level in all the treatments at both localities, (Table 4). The most important and characteristic components in tea are the catechins, a class of polyphenols that range between 12-24 % (Chen & Yu, 1994). During the present attempt two catechins, EGC and EPC of the four major were studied. Catechins principally affect the quality of made tea (Goto *et al.*, 1996; Beecher *et al.*, 1999). During the processing of black tea, about 90 % of catechins undergo enzymatic oxidation to products, directly responsible for the characteristic colour of tea brews, their astringency and unique taste (Chen & Yu, 1994).

Present study revealed that apart from the genetically controlled factors determining the inherent quality of the made tea, the environmental conditions affecting the natural growth of the tea plants, leaf characters and their composition have great effect on the quality of tea. Both soil and climate affect quality. In particular, climatic conditions including temperature, relative humidity and rainfall are important. Moreover, all daily and seasonal variations play an important role in determining quality. Conditions causing an uneven growth of the tea bush prevail mostly in Battal, where an irregular-cropping season due to variations in moisture and temperature tends to improve the quality of tea (Teranishi & Hornstein, 1995). Tea grown at higher altitude as evident from the present investigation, have higher concentration of tea leaf constituents *i.e.* EGC, EPC and caffeine compared with the inferior quality tea produced during the rainy season (Hilton *et al.*, 1973; Cloughly, 1978-79).

This study apparently suggests that in Battal, due to cold and dry weather, growth is also slow as temperature may be as low as 2°C. Slow growth and lower temperature favors quality tea production. At NTRI, there is a well-distributed rainfall and uniform temperatures for most of the year resulting in an even growth and regular cropping, producing high yield and inferior quality tea (Goswani & Barbora, 1994).

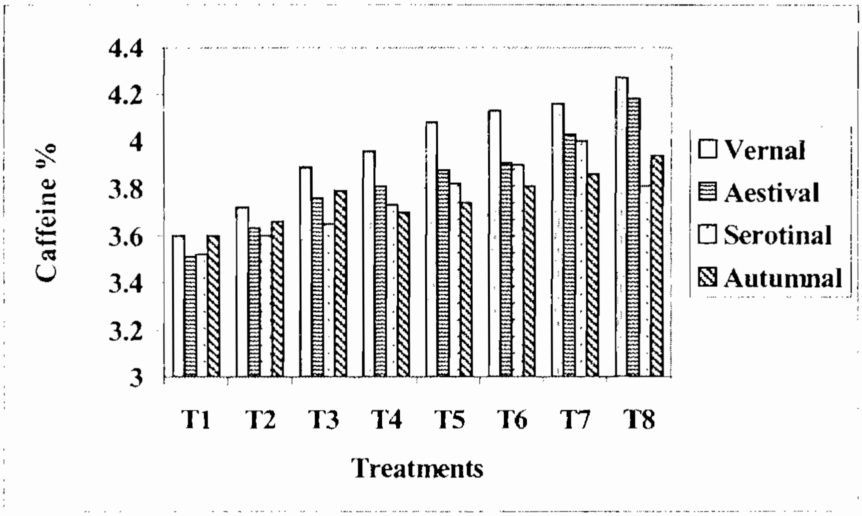

Fig. 4a. Variations in the Caffeine % in tea leaves at NTRI during different seasons under different N treatments at an altitude of 1000 m.

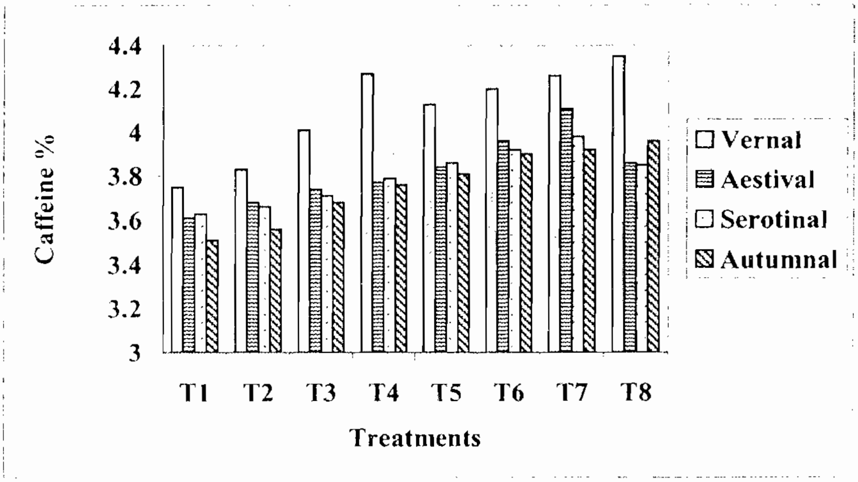

Fig. 4b. Variations in the Caffeine % in tea leaves at Battal during different seasons under different N treatments at an altitude of 1500 m.

The EGC and EPC quantity was highest in the first flush in vernal season at both the experimental sites, which may be attributed to the unfavorable climatic conditions and preceding dormant winter spell. In the periods of regular flush *i.e.* aestival and serotinal seasons, the quantity of EGC and EPC are steady as described by Cloughly (1978–79). Nitrogenous fertilizer being the main nutrient is said to affect the quality apart from increasing the yield. In this study, with the increase of nitrogen nutrients, chemical constituents of leaf increase and their response is positive. As nitrogen treatment exceeds a certain upper limit (180 kg/hec/annum; Hilton *et al.*, 1973), the response to nitrogen application, at first, remains steady and then it becomes negative and further increase of nitrogen deteriorates the quality (Scharf *et al.*, 1986). The response of EGC and EPC is positive up to T_4, beyond which their quantity drops as described by Hilton *et al.* (1973). However, Malenga (1987) described that upper limit of nitrogen application required to achieve good quality is 100 kg/hec/annum. Rahman *et al.* (1978) mentioned that high levels of nitrogen increases the strength of liquor due to increased tea leaf constituents.

In addition to changes in growth, high altitude is required in the production of quality and outstanding flavor. Our results of tea quality grown at higher altitudes are similar to those of Teranishi & Hornstein (1995) and Cheng (1995), where it is ascribed to the lower temperatures at higher elevation and to the correspondingly slower growth. The up country teas, as described in the present findings, contain higher amounts of poylphenol and caffeine, highly appropriate for black tea processing (Eden, 1976; Teranishi & Hornstein, 1995). The alkaloid, caffeine contributes to the briskness and creaming properties of tea and accounts for 5–10 % of the solid material extracted from tea leaves (Teranishi & Horstein, 1995). Present attempt suggests that the concentration of caffeine and its metabolism in tea leaves is dependent on climatic factors and agronomic practices. It also varies with the altitude. Caffeine quantity were found to be highest in rapid growing season and in the early flush. It reached the maximum in vernal season while remained constant during rest of the seasons. Similar observations have been made by Suzuki *et al.* (1990) and Teranishi & Hornstein (1995).

Increasing nitrogen treatments have pronounced effects on the level of caffeine content. During this research, caffeine concentration was found to be directly proportional to the increasing nitrogen treatment and the highest value was achieved at maximum nitrogen treatment. High caffeine content, however, produces creaming tendency in the tea liquor and hence makes it inferior in quality (Owour *et al.*, 1987a). Very high quantity of caffeine with respect to quality is an undesirable attribute. Standard levels of caffeine in young shoots of tea leaves lie in the range of 3–5 % on a dry weight basis (Robertson, 1992) and our findings suggests that at NTRI the range is 3.51–4.27 % and at Battal, quantity of caffeine ranged between 3.51–4.35 % on a dry weight basis. Our findings are in accordance with Owour *et al.* (1987a, 1990) in which they revealed that tea grown at higher altitudes contains more caffeine and hence is of good quality. Tea produced at Battal in vernal season contained comparatively more quantity of total polyphenol and it suited for the processing of black tea. Study of different nitrogen applications to tea plant demonstrates that the optimum treatment to obtain better quality tea is T_4 beyond which use of high doses of fertilizer will be uneconomical.

Conclusion

Vernal season produced tea of best quality at Battal than at NTRI. The Nitrogen application @ 180 kg/hec/annum was found desirable for enhancing tea quality, beyond which it may cross the upper critical limit so the attributes of the crop may crumble. The altitude has marked effect on the quality of tea thus grown more, up-country tea is always preferred for quality over tea grown at low elevations. Commercially, tea grown in Pakistan is suitable for black tea processing. The quantity of caffeine in tea samples fall within the range of standard value limits. From medical perspective, tea produced in our country is recommendable for oral ingestion without posing any threat to the health and beyond the degree of addiction. Tea grown in Pakistan is found in no way inferior to the tea produced anywhere else in the world as far as the determined constituents are concerned. By maintaining the processing according to the recommendations of international standards delineated by international tea trade laws in this regard, we can produce tea of outstanding flavour and taste.

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