Original Research Report

DYNAMICS OF CAFFEINE AND POLYPHENOL IN PLUCKABLE TEA SHOOTS OF TEN CLONAL AGROTYPES CULTURED AT BTRISS, ODAHLEA, BANGLADESH.

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ABSTRACT

Caffeine and polyphenol status in pluckable shoots of ten clonal agrotypes (*viz*; TV_1 and BT_1-BT_9) of 20 years old tea plants cultured under same agro-climatic conditions at BTRISS, Odahlea, were studied on three plucking periods (*viz*; increasing, peak and decreasing) in a full tea year. The soil of tea plantation sites was assessed to be sandy-clay-loam, acidic and infertile. Estimated caffeine and polyphenol contents ranged from 27.57-37.39 mgg^{-1} and 117.89-211.39 mgg^{-1} respectively in all clonal agrotypes. Maximum values for caffeine and polyphenol contents were measured on increasing and peak period respectively in each clonal agrotype. It concludes that caffeine and polyphenol contents of pluckable tea shoots of ten clonal agrotypes varied markedly with plucking periods compared to agrotypes and can be ranked superior in the order of BT_2 for caffeine and BT_7 for polyphenol contents. This study suggests the mechanism of manufacturing of high, medium and low graded tea of caffeine and polyphenol with season irrespective to agrotypes.

Keywords: Agrotypes, tea twigs, caffeine, polyphenol, plucking periods.

INTRODUCTION:

Caffeine is an important alkaloid. Tea and coffee are largely used as popular drink where caffeine is one of the most desired components. But tea is undoubtedly recognized as the world's most widely consumed beverage other than water (Stagg and Millin, 1975; Khan and Mukhtar, 2007). Chemically caffeine or 1,3,5 trimethyle xanthenes ($C_8H_{10}O_2N_4$) is an alkaloid, along with other two isometric dimethyle xanthines namely theobromine and theophylines which are present in low amount. Caffeine is well known as central nervous system stimulant (Stagg and Millin, 1975). Caffeine also contributes to the characteristic taste of a tea infusion, forming a physico-chemical complex with polyphenols, principally of the theaflavin class (Roberts, 1962; Collier *et al.*,1972). Complex formation itself possesses the desirable taste described as "tangy astrigency" (Sanderson *et al.*, 1976). This complex contributes to the formation of the coloured precipitation or 'cream' when a tea liquor is allowed to cool (Roberts, 1962; Smith, 1968). The degree of cream formation is largely dependent upon the caffeine content (Smith, 1968) and is used by professional tea tasters as an indication of quality and hence for the evaluation of a tea standard. Tea polyphenol or tea tannin also plays an important role in the quality of tea. Some polyphenol compounds are oxidised and condensed to theaflavin, which are thought to be responsible for colours, brickness and taste, and therefore are

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considered to be important for the quality of black tea.

With this view in mind, an intensive and extensive research on qualitative and quantitative estimation of caffeine and polyphenol status of pluckable tea shoots was carried out on three plucking periods in one tea year.

MATERIALS AND METHODS:

An experimental site namely section-7 with ten cbnalagro-types (*viz*; TV_1 and BT_1-BT_9) of tea -*Camellia sinensis* (L.) O.kuntze, cultured at BTRISS (Bangladesh Tea Research Institute, Sub-station), Odahlea, in 1985, was selected for the present study. The study site is situated about 50-52 m high from the sea level at an altitude of 20°40'-20°42' N and latitude of 92°4'-92°6' E on the eastern hilly side near the coast of Bay of Bengal. The area embraces mean annual rainfall, temperature, relative humidity and light intensity of 3200mm, 25°C, 90% and 3200 lux respectively. There is a clear variation of six seasons in a year with marked change in climate and soil. The experiment was designed in an area of 2 hector on CRD method and the site was divided into 5 stands. Each stand consisted of two agrotypes and each agrotype comprised of twelve rows of fifty plants. The whole site is situated in the valley of hills at an altitude of about 50m with good drainage condition. The whole tea year was divided on the basis of production and plucking intensity into three plucking periods *viz*; P_1 - increasing (May-July), P_2 - peak (Aug.- Oct.) and P_3 - decreasing (Nov.-Jan.) (Muttalib- 2000 – per commn.). Pluckable twigs were graded as high (high caffeine and medium polyphenol), medium (medium caffeine and high polyphenol) and low (low caffeine and low polyphenol) quality tea on the basis of quantity of caffeine and polyphenol on increasing, peak and decreasing periods respectively.

Soil samples from each stand were collected in triplicate using soil augar from two different depths (*viz.*top soil (1-25cms) and sub soil (26-50cms) on early monsoon, monsoon and late monsoon (Chowdhury and Rahman, 1991, 1994, Chowdhury and Alam, 2001). All sieved soil samples were dried in oven at 105°C for 72 hours and used to determine major physicochemical properties (*viz.* ST, OC, OM, p^H, EC, CEC, N, P, K, Ca and Na) except that of SMC where it was determined after 72 hour of rainfall gravirnetrically (Misra, 1968). Soil texture was determined by Bouyoucus Hydrometer Method and named after soil texture class triangle (USDA, 1951). Soil OC and OM were determined by Wet-Oxidation Method (Walkley and Black, 1934). p^H and EC were determined based on 1:2 soils and water suspension using calibrated digital p^H meter and conductivity meter respectively. CEC was extracted with 1M KCl and determined by Micro-Kjeldahl Distillation Method (Jackson, 1973). Total nitrogen of soil was extracted with mixed catalyst (H_2SO_4+$CuSO_4$) and determined by Micro-Kjeldahl Distillation Method (Jackson, 1958). Available P, K, Ca and Na were extracted with 1M-ammonium acetate-NH_4OAC. Soil phosphorus was determined spectrophotometrically (Blue Colour Method-Jackson, 1958) and K, Ca and Na were measured by Flame Photometer- Method (Jackson, 1958).

Ten plants of each clonal agrotype were earmarked randomly from each stand to collect shoot samples (two young leaves and one bud) for determining the qualitative and quantitative estimation of caffeine and polyphenol status of pluckable shoots of tea in a full tea year. Twig caffeine contents were determined by Chloroform Method (Annon-BDSS,1975) and polyphenol content was estimated by Spectrophotometer Method (Molla,1981; Chaudhury,1993; Alam,2000). Statistical analyses were done according to student's t-test and CV% was calculated as (SD/Mean value) x 100 (Tarique, 1997).

RESULTS:

The results as shown in Table-1 reveal that physico-chemical properties of soils of study area changed with profiles, stands and seasons. But the variations were not remarkable ones. The average value of SMC, OC, OM, EC, CEC and p^H were found to be 11%, 0.5%, 0.9%, 158 $mscm^{-1}$, 7 $meqg^{-1}$ and 5 on early monsoon, 15%, 0.6%, 0.9%, 120 $mscm^{-1}$, 7 $meqg^{-1}$ and 5.5 on monsoon and 14%, 0.5%, 0.9%, 69 $mscm^{-1}$, 7 $meqg^{-1}$ and 5.5 on late monsoon respectively.

Table1: Change of physico-chemical properties of soils on three plucking periods at BTRISS, Odahlea, Bangladesh.

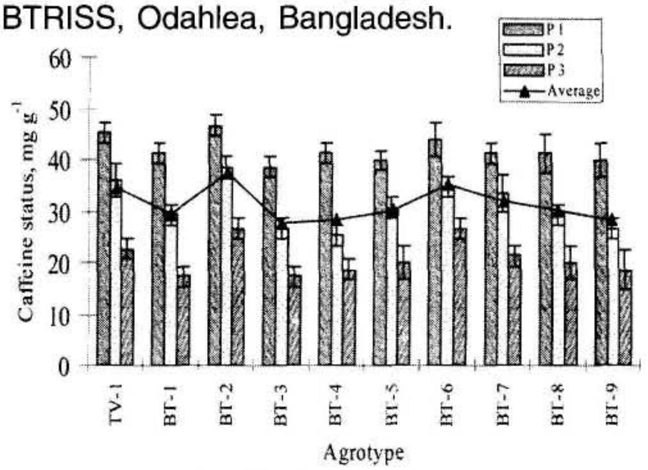
Period	Moisture content (%)	Texture	p^H	EC μscm^{-1}	OC (%)	OM (%)	CEC $meqg^{-1}$	Total N (μgg^{-1})	Available nutrients (μgg^{-1})			
									P	K	Ca	Na
P_1	11.45	SCL	4.83	158	0.51	0.88	7.25	810.7	9.90	10.60	33.40	33.10
P_2	14.62	SCL	5.65	119.5	0.55	0.94	7.48	814.2	10.01	13.84	41.26	33.58
P_3	13.65	SCL	5.47	68.5	0.49	0.84	6.77	714.1	7.86	13.26	42.80	34.85
CV%	12.27		8.11	38.93	5.91	5.68	5.05	7.29	13.08	13.75	12.88	2.67

Legend: Increasing (P_1)-Early monsoon; Peak (P_2)-monsoon; Decreasing (P_3)-Late monsoon.
Data are the average mean of three samples of nine replicates.

The average value of nutrients such as total N, available P, K, Ca and Na were found to be 811, 10, 11, 33 and 33 mgg^{-1} on early monsoon, 814, 10, 14, 41 and 34 mgg^{-1} on monsoon and 714, 8, 13, 43 and 35 mgg^{-1} on late monsoon respectively. In general, the soil was assumed to be sandy clay loam, acidic and infertile. The calculated CV% for any of the above mentioned studied parameters did not exceed beyond 14% except that of EC where it was estimated to be 39%.

The results as shown in Figure-1(a) exhibit that caffeine content of pluckable shoots was found to vary with agrotypes and plucking periods. On increasing period caffeine content varied from 38.67 mgg^{-1}(BT_3) to 46.670 mgg^{-1}(BT_2). On peak period it varied from 25.33 mgg^{-1}(BT_4) to38.67 mgg^{-1}(BT_2). On decreasing period it varied from 17.33 mgg^{-1} (BT_1, BT_3) to 26.67 mgg^{-1}(BT_2, BT_6).The highest average value of caffeine content (average of three plucking periods) was found to be 37.34mgg^{-1} in BT_2 and the lowest value was found to be 27.56 mgg^{-1} in BT_3 and showed the following sequenceBT_2>BT_6>TV_1>BT_7>BT_5>BT_8>BT_1>BT_9 >BT_4>BT_3.

Fig 1(a): Effect of Plucking periods on the change of caffeine status in pluckable tea shoots of ten clonal agrotypes cultured at BTRISS, Odahlea, Bangladesh.



The results also indicate that maximum and minimum caffeine contents were found on increasing and decreasing period respectively in each agrotype and showed the following sequence as increasing period>peak period>decreasing period. Correlation studies between physico-chemical properties of soil and caffeine content of pluckable shoots of ten clonal agrotypes revealed that physico-chemical properties of soil did not show any significant correlation value with the caffeine content of TV_1, BT_2, BT_4, BT_5, BT_6, BT_7 and BT_8. But total nitrogen content of soil was negative significantly correlated with the caffeine content of BT_1, BT_3 and BT_9. ANOVA of caffeine content showed significant value with both agrotypes and plucking periods (Table-2).

Table 2: Analysis of variance of caffeine and polyphenol status in pluckable tea shoots of ten clonal agrotypes and three plucking periods.

Source of variance	Degree of freedom	F-Values	
		Shoot caffeine	Shoot polyphenol
Agrotypes	10-1=9	*5.15	0.55 NS
Plucking periods	3-1=2	**40.5	*47.87
Errors	29-11=18	------	-------

Legend: * significant at 5% level, ** significant at 1%level and NS = non- significant.

with agrotypes and plucking periods. Polyphenol content ranged from 207.92 mgg^{-1} (BT_8) to 235.65 mgg^{-1}(BT_4), 246.04 mgg^{-1}(BT_1) to 315.35 mgg^{-1}(BT_7), 65.84 mgg^{-1}(BT_3) to 107.43 mgg^{-1}(TV_1) in increasing, peak and decreasing period respectively. The highest average value of polyphenol content (average of three plucking periods) was found to be 211.39 mgg^{-1} in BT_7 and the lowest value was found to be 177.89 mgg^{-1} in BT_1 and showed the following sequence as BT_7>BT_4>BT_6>BT_8>TV_1>BT_2>BT_9>BT_5>BT_3>BT_1.

The result also show that maximum polyphenol content was found on peak period and minimum on decreasing period in each agrotype and showed the following sequence as peak period>increasing period>decreasing period. Correlation studies between physico-chemical properties of soil and polyphenol content of pluckable shoots of ten clonal agrotypes revealed that physico-chemical properties of soil did not show any significant correlation value with the polyphenol content of BT_5. Available potassium content of soil was negative significantly correlated with the polyphenol content of TV_1, BT_1, BT_2 and BT_4 whilst CEC was negative significantly correlated with the polyphenol content of TV_1 and BT_1. Further, moisture content of soil was negative significantly correlated with the polyphenol content of BT_6, BT_7, BT_8 and BT_9 whereas sodium content was negative significantly correlated with the polyphenol content of BT_6, BT_7 and BT_8. Organic matter of soil was negative significantly correlated with the polyphenol content of BT_3 only. ANOVA of polyphenol content showed significant value with plucking periods but did not show any significant value with agrotypes (Table-2). It seems that all these ten agrotypes do not respond to the same soil factors at the same rate in the production of caffeine and polyphenol contents of pluckable shoots which may be attributed to the physiological differences of agrotypes.

DISCUSSION:

Choudhury (1989) reported some of the characteristics of good tea soils which are as follows: texture-sandy loam; organic carbon rich-2%, medium-1% and poor-below-1%, p^H 4.5-5.8; total N-not less than 0.1%, available P-10 mgg^{-1}, K-80 mgg^{-1} and Ca-90 mgg^{-1} respectively. The physico-chemical properties of studied experimental plots were nearly the same as those reported by Choudhury (1989). The present results show that the studied soil was sandy clay loam, acidic and infertile.

The results show that both the caffeine and polyphenol contents varied with agrotypes and plucking periods. Maximum caffeine content was found on increasing period and minimum on decreasing period. Maximum polyphenol content was determined on peak period and minimum on decreasing period. The highest average values of caffeine and polyphenol content were determined in BT_2 (37.34 mgg^{-1}) and BT_7 (211.39 mgg^{-1}) and the lowest values were determined in BT_3 (27.56 mgg^{-1})and BT1 (177.89 mgg^{-1}) respectively. The quantity of these elements was found to be reasonably high. Tea generally contains 10-50 mg of caffeine and 150-180 mg of polyphenol (Cloughley, 1981). Total polyphenol content ranges up to 18% in black tea leaf while it ranges up to 23% in green tea of Australia (Caffin *et. al.*, 2004). The range of caffeine content in Bangladesh tea is 33-48 mgg^{-1} and that of polyphenol is 221-310 mgg^{-1} (Choudhury, 1990 and Chowdhury and Alam, 2001). The caffeine content of tea is reported to be influenced by seasonal, genetic, agronomic and cultural factors and the highest levels of caffeine are produced when shoot growth rates are most rapid (Cloughley, 1982). Chowdhury and Rahman (1989) reported that the amounts of caffeine in BT_1 grown at Odahlea tea estate were measured to be 48 mgg^{-1}, 29 mgg^{-1} and 16 mgg^{-1} on increasing, peak and decreasing periods respectively. Caffeine decreases progressively with seasons in freshly plucked tea shoots during a tea year (Wood *et. al.*, 2006). Total and extractable caffeine contents of black tea samples gradually decrease during a tea year after a rapid increase in the early season in Argentinean teas (Malec, 2006). Polyphenols constitute about 15% w/w of Black tea where the composition varies with variety of tea, its geographical origin, environmental conditions and agronomic situations (Stagg and Millin, 1975). In the present study, the measured values of both caffeine and polyphenol are comparable with the findings of Stagg and Millin (1975), Cloughley (1981, 1982), Chowdhury and Rahman (1989), Choudhury (1990) Chowdhury and Alam (2001), Caffin *et. al.* (2004), Wood *et. al.* (2006) and Malec (2006). This study concludes that both caffeine and polyphenol status of pluckable shoots of tea in ten clonal agrotypes varied markedly with plucking periods compared to agrotypes where maximum caffeine and polyphenol contents were measured on increasing and peak periods respectively, and can be suggested to screen out all the studied agrotypes as superior in the order of BT_2 for caffeine and BT_7 for polyphenol contents.

The results of the present study further state that early monsoon (increasing plucking period) favours the production of tea with high caffeine and low polyphenol content whilst monsoon (peak plucking period) favours the production of tea with medium caffeine and high polyphenol content. But late monsoon favours the production of tea with both low caffeine and polyphenol contents. This study suggests a mechanism of manufacturing of high (high caffeine–medium polyphenol), medium (medium caffeine – high polyphenol) and low (low caffeine – low polyphenol) graded tea with seasons irrespective to agrotypes which can be assessed and ranked in the order of early monsoon>monsoon>late monsoon for caffeine and monsoon>early monsoon>late monsoon for polyphenol respectively. It can further be concluded that the harvested tea of early monsoon (increasing period) is better compared to the products of monsoon and late monsoon in term of caffeine quality. Whilst the tea of monsoon (peak period) is better in term of polyphenol content compared to early monsoon and late monsoon. This fact has been verified in three alternate tea years on the tea plantation of same experimental plots since 1998. So, season is regarded as one of the major controlling factor for both quality and quantity of tea in terms of caffeine and polyphenol contents. The tea growers may be suggested to take care of this fact in the plucking-manufacturing of brand teas of three different grades to suffice the demand of consumers from both favorite and medical view points.

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