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Effects of climatic factors on antioxidant quality of tea (*Camellia sinensis*) in North Bengal

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ABSTRACT

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the important contributors to the growing economy of North Bengal. The phytochemical and therapeutic qualities of tea prominently altered recently due to continuous change in climatic conditions of North Bengal. The nutraceutical quality of tea is largely dependent on antioxidant attributes. For investigating the relationship between antioxidant attributes and climatic factors, the present field experiment was conducted in 18 tea gardens of Terai, Dooars and Darjeeling Hills of North Bengal during 2012–17. Antioxidant and phytochemical analysis was performed in three different harvest seasons, viz. March, June and December. Phenolic compounds including catechins were low during winter months, and then gradually increased up to warmer spring season. In stress condition, tea plants biosynthesized more flavonoids and high flavour index in Darjeeling hills. However, free-radical scavenging and metal chelating activities were found to increase from spring to winter season. The data were pooled for PCA analysis to determine the relationship between seasonal variations and tea antioxidant quality in each region of North Bengal separately. Different attributes of climatic factors significantly correlated with antioxidant quality and bioactive compounds. Variation in climate is reportedly affecting the antioxidant quality of tea and its shifting pattern grossly influences the key phytochemicals responsible for the flavour of tea.

Keywords: Antioxidants, PCA, Phytoconstituents, Tea

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the world's oldest panaceas. Tea is consumed in almost every country around the world and has reached a ceremonial status as a social and medicinal beverage (Helliwell *et al*. 1999). Antioxidants are almost universal in normally consumed herbal food products, they are pre-existing compounds in the form of natural secondary metabolites; sometimes also required during processing of synthetic antioxidants. As long as they are consumed in moderate concentration, natural antioxidants have been proven to have several positive health effects as compared to their synthetic counterparts (Daniel 1986). Recently it has been conceptualized that ideal food/beverage should have all the required nutrients as well as nutraceutical and antioxidant principles (Danesi 2009). The bioactive food component refers to non-essential biomolecules that exhibits the capacity to modulate several metabolic processes that result in the promotion of better health.

The variation of chemical content, as well as antioxidant potential present in tea shoots in various climatic factors of North Bengal, has not been studied so far. In this context, the study was designed for in-depth analysis of antioxidants and region wise climatic factors along with the quantity of bioactive phytochemicals of under-explored tea of Darjeeling Himalaya, Dooars and Terai. The results may be helpful to understand the effect of geographical and climatic factors on biological activity of this useful plantation crop.

MATERIALS AND METHODS

The experiment was conducted during 2012–17. Tea buds and young leaves were collected from mature tea bushes along with soil samples and climatic data of different selected tea gardens situated at Dooars, Terai, and Darjeeling Hills of North Bengal.

Study area: The study area included six tea gardens each from the Terai [New Chamta Tea Garden (TG), Matigara Tea Estate (TE), Hansqua TG, Gayaganga TG, Motidhar TG and Sayedabaid TE], Dooars [Samsing TG, Ghatia TG, Nagragata TG, Indong TG, Debpara TG and Binnaguri TG] and Darjeeling Hill (Soom TG, Happy Valley TG, Chamong TG, Singtom TG, Phubsering TG and Phuguri TG). The geo-climatic regions of West Bengal with a geographic area of 5.19 lakh ha; within which 115,000 ha produces tea included Terai, Dooars and Darjeeling Hills stretching between 26°12'–27°07'N latitude and between 88°05'–89°21'E longitude; the altitude ranged from mean

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sea-level to the highest mountain ranges (1400 m–50 m).

Climatic and geographic distribution data collection: The Climatic data like aerial temperature (maximum and minimum in °C), relative humidity (%), precipitation (mm), rain day (number), wind speed (km/h), atmospheric pressure (mm/Hg), cloud percent, UV Index (1 UV Index = 25 milli Watts per square meter), sunshine hours, sunny day, visibility (km) along with altitudes (m) geographical locations (latitude and longitude) were collected from the records of Central Tobacco Research Institute (CTRI) at Coochbehar, Darjeeling Tea Research Centre (DTRC), and Meteorological station at Gungaram Tea Garden and also Tea gardens' individual data sources.

Plant materials: A pilot survey was conducted from the data sources of the tea gardens to document the distribution of tea plant variety and their age (20–50 years). The plant specimens [different selected cultivars of *Camellia sinensis* (L.) O. Kuntze] i.e. apical bud and two fresh tea leaves were randomly collected from the different cultivated sections of the tea garden for assessment of antioxidant attributes and phytoconstituents.

Preparation of plant extracts: Fresh tea leaves (two leaves and a bud) were crushed in mortar and pestle with methanol. The methanol was completely removed by vacuum rotary evaporator at 50°C. One part of the crude extract was used for experiment and other parts were freeze-dried. The powder was stored at 4°C and used for further investigation.

Determination of free radicals scavenging activities: Free-radical scavenging activities like DPPH, Superoxide, Nitric Oxide (NO), Hydroxyl (HO), Anti-lipid Peroxidation (ALP), $ABTS^+$ radical scavenging and Metal Chelating (MC) activities were determined following standard protocol (Mandal *et al*. 2009). Scavenging and chelating activities were measured as Inhibition Percentage (I %).

$$I\% = [(A_0 - A_i)/A_0] \times 100$$

where A_0, Absorbance in Control; A_i, Absorbance in Sample. IC_{50} of scavenging activity was defined as concentration of tea extract where half of the reduction in radical scavenging was achieved.

Estimation of phyto-constituents: Bioactive phytochemicals like total phenolic compounds [TPC] (Folin and Ciocalteu 1927), flavanol, quinones, tannins (Mahadevan *et al.* 1986), and Flavour Index (FI) (Masoud *et al*. 2006) were determined by using standard spectrophotometric methods. The analytical estimation of epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) was carried out using reverse phase (C_{18} column) high-performance liquid chromatography in isocratic mode as per method prescribed by Misra *et al*. (2016).

Statistical analysis: The data were pooled in triplicate. Relationships between different phytochemicals and antioxidant traits, a principal component analysis (PCA) based on the correlation matrix was calculated using Multivariate Statistical Package (MVSP 3.1). Smith's Statistical Package version 2.5 (prepared by Gary Smith, CA, USA) was used for determining the IC_{50} values of

Table 1 Climate attributes of three geographical regions of North Bengal and their variation with season [Mean ± SD]

Season	Temp. max. (°C)	Temp. min. (°C)	Daily rainfall (mm)	Rain day (nos.)	Wind speed (km/h)	Atmos. pressure (mm/Hg)	Cloud (%)	RH (%)	UV index	Sunshine hours	Sunny days	Visibility (km)
Terai												
March	34 ± 04	21 ± 04	10.80 ± 6.70	05 ± 01	7.8 ± 2	1013.1 ± 21.91	08 ± 03	32 ± 03	06 ± 0.5	122.5 ± 5.7	25 ± 2	6 ± 1
June	35 ± 03	27 ± 05	397.72 ± 99.80	30 ± 05	7.4 ± 3	1006.1 ± 11.23	43 ± 07	74 ± 09	07 ± 0.8	129.3 ± 25.2	00 ± 0.0	4 ± 1
December	28 ± 05	15 ± 06	04.00 ± 0.00	00 ± 00	4.9 ± 2	1015.9 ± 34.21	06 ± 02	64 ± 03	06 ± 0.6	93.4 ± 2.3	31	6 ± 1
Dooars												
March	37 ± 05	21 ± 05	53.93 ± 05.8	9 ± 01	7.4 ± 2	1012.4 ± 19.42	29 ± 07	70 ± 08	6 ± 0.3	123.8 ± 6.3	21 ± 3	6 ± 2
June	35 ± 03	26 ± 05	563.96 ± 83.21	30 ± 04	4.5 ± 3	1005.2 ± 21.34	40 ± 08	82 ± 09	7 ± 0.7	112.5 ± 17.4	00 ± 0.0	5 ± 1
December	30 ± 04	17 ± 04	6.3 ± 01	5 ± 01	4.7 ± 2	1015.2 ± 25.13	59 ± 05	15 ± 04	6 ± 0.4	86.3 ± 4.3	25 ± 4	6 ± 1
Darjeeling Hills												
March	28 ± 03	18 ± 05	39.78 ± 05.9	10 ± 02	8.3 ± 1	1014.4 ± 23.10	09 ± 02	31 ± 06	5 ± 0.3	122 ± 5.8	19 ± 2	6 ± 2
June	31 ± 04	24 ± 06	490.13 ± 76.43	30 ± 05	5.6 ± 1	1007.3 ± 17.32	41 ± 07	73 ± 09	6 ± 0.6	151 ± 12.31	00 ± 0.0	5 ± 1
December	23 ± 06	14 ± 03	24.92 ± 8.61	11 ± 04	5.6 ± 2	1016.6 ± 15.30	11 ± 03	55 ± 03	5 ± 0.4	90.8 ± 15.6	25 ± 6	6 ± 2

antioxidants and their standard error of estimates (SEE).

RESULTS AND DISCUSSION

North Bengal is a land of wide scale climatic and seasonal variations (Table 1), affording scope for much diversity in tea cultivation. Seasonal impact of climatic factors in North Bengal are important because the fluctuating behavior influences the polyphenol content, sensory quality and antioxidant profile of tea leaves. In this study, climatic variation of phenolic compounds (mainly EGCG and ECG) and antioxidant properties in fresh tea shoots consisting of one apical bud and two adjoining leaves were investigated in three harvest season: March, June and December. The total phenolic compounds including EGCG and ECG were comparatively lower in winter months of December, thereafter increased throughout the warmer months up to March and decreased drastically during the rainy season from June to September (Table 2). The great difference in tea shoots in terms of total phenolics at different harvest time is believed to be the effect of change of climatic attributes. Tea gardens of North Bengal receive less sunlight in December. This might affect the biosynthesis of total phenolics and flavonoids. Conversely, in the month of December during winter season, the difference between day and night temperatures was also higher and the rainfall was irregular. In this stress condition, tea plants produced more phenolics, and high flavour index (FI), particularly at Darjeeling Hills. On the basis of information given by Mahanta *et al.* (1992), the differences in total phenolic levels in fresh tea shoots harvested at different months in North Bengal may not be just a temperature effect but also day length and sunlight effect. Our results are in agreement with the findings of Yao *et al.* (2005), who found that more phenolics were accumulated in warmer months in the tea shoots. In general free radical scavenging and metal chelating activities were found to increase from 1st harvest (March) to last harvest (December) (Table 2). Apparently, there was a strong relationship between the free radical scavenging activity and total phenolics in all harvest times.

To study more about the relationship between climatic factors, profiling of phytoconstituents, and antioxidant attributes, PCA was performed in each region of North Bengal. The 26 principal components were chosen on the basis of their eigen values, explaining the total data variance for Dooars, Terai and Darjeeling hill. In almost all cases, climatic attributes were heavily loaded on the second principal component (PC2) and clustered as evidenced by the correlation matrix analysis.

In Dooars region (Fig 1), $ABTS^+$, hydroxyl radical scavenging and anti-lipid peroxidation were clustered in left co-ordinate of PC2 with rainfall, rainy day, UV index which indicated that these antioxidant attributes were closely controlled by climatic factors, but metal chelating, nitric oxide, Flavour Index and DPPH free radical scavenging attributes were loaded on top coordinate of PC1 and were clustered in opposite domain with cloud percentage indicating that the enhancement of cloud is important for reducing percentage values of these scavenging

Table 2 Free radical scavenging and metal chelating activity (in terms of IC_{50} values) along with quantitative phytochemical profile (Mean ± SD) of fresh green tea leaves of North Bengal

Season	DPPH	NO	OH	SO	ALP	$ABTS^+$	MC	EGCG%	ECG%	FI	TP	FLAV	QUIN
Terai													
March	95.36 ± 5.2	12.50 ± 2.2	93.20 ± 6.1	82.40 ± 3.8	74.50 ± 6.3	68.20 ± 6.2	84.20 ± 8.2	4.46 ± 0.2	1.93 ± 0.1	0.24 ±0.05	25.78 ± 2.6	13.54 ± 1.9	0.14 ± 0.05
June	78.21 ± 4.8	14.60 ± 2.8	92.10 ± 4.2	70.20 ± 4.3	66.30 ± 4.8	60.40 ± 7.1	86.30 ± 4.4	2.52 ± 0.4	0.94 ± 0.2	0.15 ±0.08	20.89 ± 4.7	9.45 ± 2.1	0.58 ± 0.07
December	84.25 ± 6.1	12.30 ± 1.9	97.50 ± 5.5	74.30 ± 5.2	68.90 ± 4.2	65.70 ± 5.9	85.40 ± 5.2	3.93 ± 0.5	1.75 ± 0.3	0.21 ±0.05	26.88 ± 5.2	12.61 ± 1.9	0.12 ± 0.06
Dooars													
March	84.25 ± 4.8	15.60 ± 3.1	95.60 ± 8.6	81.30 ± 6.4	62.30 ± 2.4	54.20 ± 6.3	88.52 ± 7.6	4.66 ± 0.8	2.25 ± 0.45	0.17 ±0.08	28.45 ± 4.9	14.91 ± 1.5	0.73 ± 0.08
June	89.62 ± 7.8	14.20 ± 2.9	96.30 ± 7.5	78.90 ± 7.2	78.52 ± 3.4	56.40 ± 4.8	85.40 ± 6.9	4.93 ± 0.6	0.98 ± 0.28	0.13 ±0.07	22.73 ± 5.2	12.41 ± 2.0	0.16 ± 0.05
December	58.94 ± 5.5	11.80 ± 2.2	95.78 ± 6.8	85.20 ± 6.4	65.32 ± 3.9	54.65 ± 5.8	78.98 ± 6.8	4.11 ± 0.9	2.61 ± 0.74	0.14 ±0.05	26.15 ± 6.2	12.61 ± 1.8	0.13 ± 0.04
Darjeeling Hills													
March	85.69 ± 4.7	17.80 ± 3.4	95.60 ± 5.8	84.50 ± 6.2	62.32 ± 7.8	74.50 ± 3.5	87.45 ± 5.6	5.28 ± 0.6	2.34 ± 0.35	0.12 ±0.01	27.58 ± 0.28	14.75 ± 0.56	0.11 ± 0.03
June	85.65 ± 6.8	14.50 ± 2.1	98.60 ± 6.4	77.12 ± 5.8	74.25 ± 6.2	78.60 ± 4.6	89.65 ± 4.7	3.73 ± 0.7	0.98 ± 0.47	0.14 ±0.04	24.69 ± 0.29	14.30 ± 0.42	0.63 ± 0.02
December	92.36 ± 5.8	14.65 ± 3.2	96.30 ± 6.2	78.00 ± 6.4	74.25 ± 5.4	74.20 ± 5.6	84.50 ± 4.3	4.94 ± 0.5	2.67 ± 0.31	0.15 ±0.01	26.32 ± 0.46	14.65 ± 0.38	0.74 ± 0.01

components. ECG values also verified with correlation matrix analysis. From correlation matrix it was also revealed that Flavour Index was directly associated with maximum temperature and sunshine hours whereas chelating capacity of the extract was directly dependent on maximum temperature, sunshine hours and inversely related with cloud appearance.

In case of Terai region, climatic variables like visibility (fog density), sunny days and atmospheric pressure were heavily loaded with total phenol content, flavonoids, EGCG content, free radical scavenging activity ($ABTS^+$) and Flavour Index; similarly nitric oxide scavenging activity was clustered with rainfall, rainy days, cloud percentage and UV Index in opposite coordinate and this has been verified in the correlation matrix test.

For Darjeeling Hill region, EGCG, ECG,Total phenol and flavonol content were highly associated with climatic factors like visibility, atmospheric pressure, and sunny days; whereas $ABTS^+$ was directly clustered with rainfall, UV Index, rainy day and cloud percentage (Fig 2), which means that these parameters were highly correlated.

It is quite difficult to assign a physiological role of the majority of plant phenolics; there is increasing evidence that a considerable number of these substances play an ecological role in plants. The flavonoid pigments are contributed to flower and fruit colour for the purposes of attracting bees and green fly to tea plants for pollination and seed dispersal.

Results of our study are in concurrence with Ghasemi *et al*. (2011) who reported that geographical and climatic condition in different regions could lead to significant differences both in the content of bioactive compounds and their bioactivity.

Warm days, long sunshine hours, high humidity, rainfall, and preferably overnight shower affect the yield of tea (Carr 1972). Square (1985) analyzed four factors like solar radiation received, amount of solar radiation intercepted by the crop canopy, efficiency of conversion of intercepted

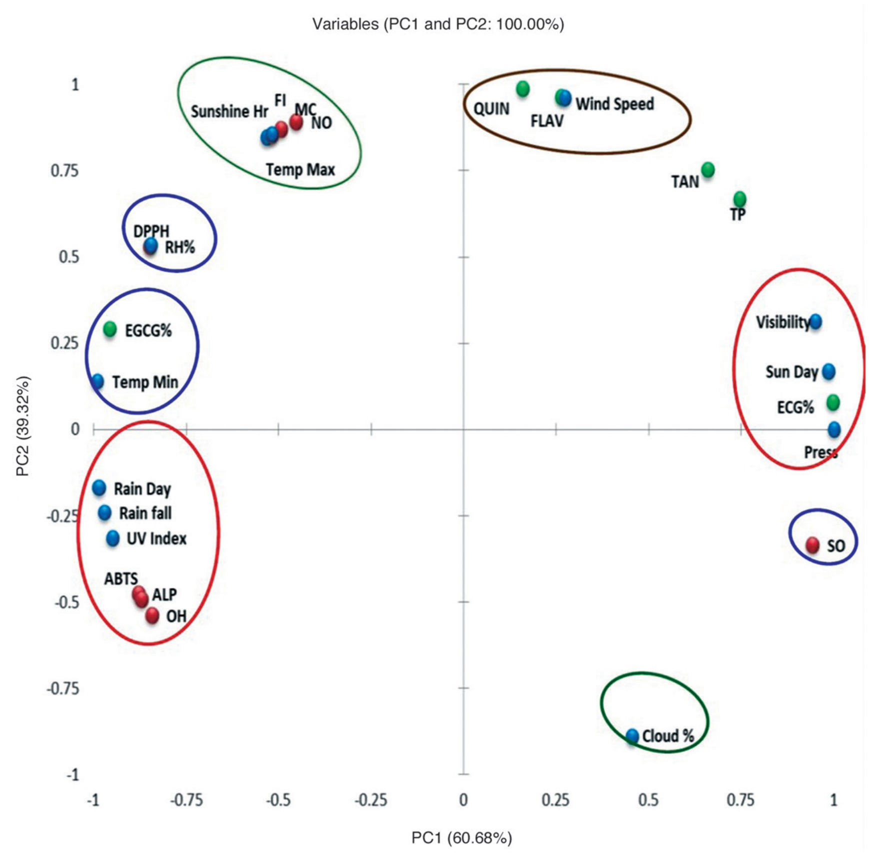

Fig 1　Plotting (PC1 × PC2) of scores and loading for the PCA of climatic factors, phytoconstituents and antioxidant attributes of Tea Garden of Dooars region.

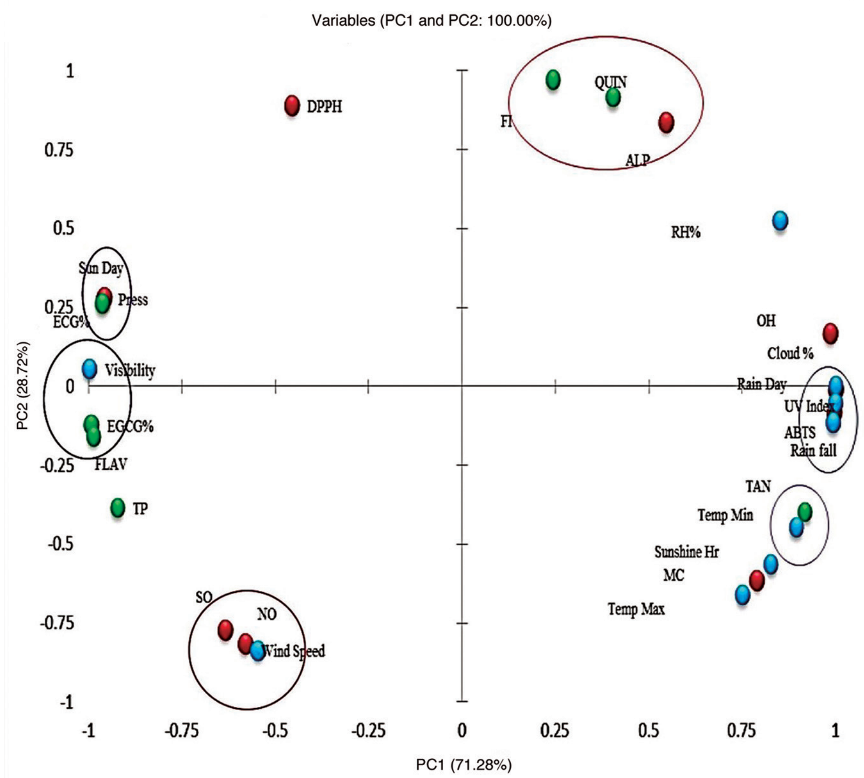

Fig 2　Plotting (PC1 × PC2) of scores and loadings for the PCA of climatic factors, phytoconstituents and antioxidant attributes of Tea Garden of Darjeeling hill region.

radiation to dry matter and fraction of the dry matter partitioned to the useful product. Minimum air temperature required to support shoot growth of tea appears to be about 14°C with an optimum range between 18°C to 30°C. Day time maximum temperature in excess of 30°C and night minimum 14°C leads to reduction in rate of tea growth, frequent hailstorms and winds are detrimental to growth (Misra *et al.* 2008). Similar finding were also observed in our experiments.

The content of phenolic compounds, antioxidant properties, and hence the quality of tea, are reportedly influenced by a variety of climatic factors, including geographical location. Less sunlight is passed through North Bengal region during mid December month (3^{rd} harvest) than the other harvest times due to heavy fog. The sunlight might affect the biosynthesis of total phenolics as phenylpropanoid derivatives are elicited by UV coming from sunlight (Sharma *et al*. 2019). The differences between day and night temperatures were also higher and the rainfall was irregular. In such a condition, tea plants produced more phenolics. It has been shown that the biosynthesis of phenolic compounds in the tea shoots can be effectively induced by stronger sunlight and length of daytime. However, further studies are required to elucidate the induction of biosynthesis of total phenolics by day length and sunlight exposure correlated with UV index.

It was revealed that shorter monsoon with decreased daily rainfall contributes to reduced tea yield in regions of North Bengal, with implications for crop management and harvesting strategies. The shifting pattern of precipitation affects the phenolic compounds and antioxidant properties of tea.

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