SUMMARY

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The present study deals with the "Evaluation of antioxidant properties of tea under various agro-climatic conditions of North Bengal". The thesis is comprised of seven chapters. At the onset of the work a brief introduction followed by literature review in concord with the line of investigation has been presented.

General aspects of tea have been included in the introduction. After that origin and history of tea along with bud dormancy, productivity etc. have been discussed.

About two-third of the world population consumes tea [*Camellia sinensis* (L.)]. In therapeutics, various kinds of tea obtained from different varieties of tea are most often employed in prophylactic purposes and in the formulation of plant derived elixir to protect human diseases. Tea productions need a combination of industry and agriculture. Northern part of West Bengal (called North Bengal) has three distinctly different tea growing regions.

The literature reviewed has been divided into several sub-heads such as, history of tea cultivation in India, taxonomy of tea, present scenario of tea cultivation, antioxidant mechanism of action, synthetic antioxidants, therapeutic and chemical characteristics of tea, assessment of free radicals scavenging activity and regional variation, quantitative variation of antioxidant activity with soil nutritional properties, agro-climatic zones of North Bengal, importance of tea cultivation, problems of tea pests**,** shade trees in tea cultivation, variation of antioxidants attribute with agro-climatic conditions and suitability, industrial processing of tea, tea as powerful and its uses in different diseases, antioxidants, metabolomics of tea polyphenols and future prospects emphasize how to restored antioxidants quality of tea.

In chapter III, deals with the location of study area, agro-climatic conditions of North Bengal includes climate, rainfall, temperature, relative humidity, Humid-prehumid ecosystem, sunshine / brightness, wind speed, rivers, soil types etc.

The scopes and objective of the study were elucidated in chapter IV. We set our objectives to examine the changes in contents of antioxidants and secondary (total

flavonoids and total phenolics) metabolites with different soil profile to search relationship among the parameters within our study area which includes twenty gardens.

The objectives of the study are as follows;

1) Study of the variation of antioxidant properties of tea in:

(i) different soil conditions

(ii) different climatic conditions

(iii) different geographical distributions

(iv) different plant varieties

(v) different processed grades

(vi) different physical conditions of processing.

2) Comparison of antioxidant properties in organically grown tea and non-organically grown tea.

3) Soil degradation pattern with the span of plantation ages and antioxidant properties of plants at different ages.

4) Study of phenolic contents in different tea.

In Chapter V, materials and methodologies have been presented with valid references wherever necessary. The different sub-heads of materials and methods are as follows:

a) Collection of unprocessed and processed tea samples

b) Record of collection of data regarding shade tree, weeds, grass, mulching materials and climatic data.

c) Collection soil sample

d) Assay phytoconstituents and antioxidants of tea viz. total phenol, flavonol, tannins, caffeine, quinone, ortho-dihydric phenol, TF, TR, TLC, FI, EGCG, ECG, antioxidants attributes viz. DPPH, nitric oxide, hydroxyl, superoxide, ALP, MC, ABTS, soil physico-chemicals attributes etc.

In chapter VI, results have been represented and analyzed with published literature. Valid discussions have been made with citation of the works related to our present results.

Field surveys were conducted and tally with existing data source of tea gardens of Terai, Dooars and Darjeeling Hills to document the distribution of tea cultivars and their ages. On the basis of the survey, ten tea cultivars were selected. Soil physico-chemicals, phytochemicals analysis, antioxidant studies of the collected samples were performed. Twenty four species of shade trees in tea plantations have been identified on various soil conditions of North Bengal region. The prevailing soil types and different soil characteristics have been recognized in the area.

Correlation matrix analysis of physico-chemical attributes of tea soil of Terai, Dooars and Darjeeling hill of North Bengal revealed that among the six tea gardens of Dooars regions, the soils of Debpara and Binnaguri tea gardens were predominantly sandy loam to clay loam in texture, whereas, the soils of Nagrakata and Ghatia were clay loam to clayey in texture. The organic carbon, organic matter and available nitrogen were found relatively higher in young aged section of Ghatia and Samsing and medium aged section of Debpara, Binnaguri, Nagrakata tea garden and aged section of Indong tea garden. Maximum potassium content was found with young section of Samsing and the lowest was with aged sections of Indong tea soil.

Soil organic carbon, organic matter, available nitrogen, phosphorus, potassium, sulphur, moisture content and chloride had a positive correlation with nutrient and physical parameter, suggesting that soil organic carbon increased the availability of nutrients under tea growing soils. Higher potassium content was found in both medium and young aged sections of all tea soils respectively. The available soil K content varied from 86.94 to 445.57 ppm.

The soil of six tea garden in Terai region; viz. New Chumta, Sayedabad, Hansqua, Gayaganga, Motidhar and Paharghumia were recorded. Physico-chemical properties of soils were strong to moderately acidic (4.10 to 5.34) with low electrical conductivity. Soils were sandy loam in texture. The clay content varied from 6.6 to 20.4%. The organic carbon, organic matter and available nitrogen content varied from 0.98 to 1.60%, 1.54 to

2.77 % and 0.14 to 0.22 % respectively in young, 0.95 to 1.80, 1.65 to 2.94, 0.13 to 0.24% in medium and 0.85 to 1.90, 1.47 to 3.28, 0.12 to 0.26% in old aged soils. The highest available nitrogen was obtained with Motidhar Tea Garden and the lowest in Gayaganga Tea Garden. Available phosphorus content varied between 8.92 to 32.55 ppm in young, 9.22 to 25.96 ppm in medium and 3.91 to 41.08 ppm in old aged soils and wide variation of soil available P might be due to the fixation of phosphorus in soil as insoluble aluminium and iron phosphate, higher acidity and poor application of rock phosphate. Available potassium content varied from the medium to high. Higher potassium content was found in both medium and young aged sections of all tea soils respectively. Available Sulphur was below recommended levels (Misra *et al*., 2009) of all section and Chloride was within limits in all sections. Moisture content was highest in medium aged section of Motidhar tea garden; this is because of using mulching in dry spell and comparatively satisfactory levels of organic matter. Soils were compared with virgin and multi-crops system of very closest area from respective tea garden, it was found that pH lies in between 4.1 to 5.50, electrical conductivity were within limits and all nutrients were satisfactory levels in Terai region.

The soils of six tea gardens in Hills of Darjeeling region viz: Phubsering, Singtom, Ambootia, Happy Valley, Soom and Chamong were recorded for three times in a year for five years. Soil pH was found to be strong to moderately acidic in nature (4.11 to 5.50), with low electrical conductivity, might be due to leaching of soluble salts by heavy rainfall. Soils under study were sandy loam to sandy clay loom in texture and the clay content varied from 8.5 to 41.20. The organic carbon, organic matter and available nitrogen content were high in all section and these variations might be due to the effect of management practices of these locations. The availability of soil phosphorus varied from 10.65 to 78.96 under low, medium and high elevations respectively. The higher availability of phosphorus under lower elevation was observed. The available soil potassium content varied from 86.94 to 445.57 ppm. Soil pH had a positive correlation with all nutrients. The phosphorus had a significant correlation with electrical conductivity, organic carbon and available potassium. The potassium had a significant positive correlation with nitrogen, phosphorus, sulphur.

Soils were compared with virgin and multi-crops systems of very closest area from respective tea garden, it was found that pH lies in between 4.26 to 5.40 and all required nutrients were within recommended (Misra *et al*.,2009) levels in hilly regions.

Phytochemical quality and free radical scavenging quality of fresh tea shoots and processed handmade tea of mostly cultivated clone of TV1, TV2, TV20, TV26, TV29, TV30, Dangri Manipuri, Tinali 17, Takda 7.8, Sundaram(B/5/63), Tingamara were assayed for characterization of cultivar.

Tea leaf Polyphenol Oxidases (PPO), which is responsible for oxidizing dihydroxy polyphenol varied from clone to clone increases by oxidative stress and have influence on soil pH content.Phosphorus deficiency resulting decreases of PPO activity.

TLC and HPLC have been used for quantification of main attributes of polyphenol *i.e.* ECG and EGCG content in fresh leaf and processed leaf and seasonal variation region wise. It has revealed that the levels of total phenolics in terms of EGCG and ECG increased throughout the warmer months from March to September.

Seasonal variation of mineral content in tea shoots with clonal variation depend, not only on the species or varieties, but also on the growing conditions such as soil and geographic condition.

Climate and agronomical factors affect both the flavonol content of green tea shoots and the composition of the resulting made tea. The present total phenolic content would indicate that there is potential to produce higher quality black tea during the December months in North Bengal. There were statistical difference among harvest times in all ten tea cultivars except TV1 ($p<0.01$). Antioxidant activity was increased from 1st harvest to 3rd harvest times in all tea cultivar. Free radical scavenging activity was found to be between 99.97-74.10%. Antioxidant activity (% free radical scavenging) of BHA (200 mg/l) was 91.18%. In previous studies conducted on tea the antioxidant activity of different tea products in different solvent was found between 56-83%. It was revealed from literature study that commonly consumed products such as tea, coffee and cocoa have possessed significant amount antioxidant activity. The results for antioxidant activity clearly outlined that tea shoots could be one of the richest sources among plants

in terms of antioxidant activity. The composition of tea shoots varies with climate, season, variety, and age of the shoot.

The chemical composition of tea shoots varies with genotype, season and cultural practices. Processed tea that is widely consumed now as a popular health drinks/beverage i.e. commercially manufactured from the young tender leaves (bud, bud + single leaf, bud + second leaf etc.) of the tea plant. This research elucidates how functional component in tea cultivar could expand the role of diet in oxidative disease prevention and treatment. There are evidences that tea constituents play therapeutic role in more than sixty different health conditions.

Certain plants show antioxidant activity with DPPH because of their phenolics constituents. Irrespective of cultivar and leaf maturation the antioxidant capacity of tea plant was significantly varied with soil physicochemical parameters. DPPH (IC_{50}) based free-radical scavenging activity was significantly correlated with soil pH, because soil pH significant affects on the availability plant nutrients and microbial activity surrounding the rhizosphere. It may be predicted that antioxidant activity restoration in tea leaves is optimized when the pH range in between 4.00 -5.50. There is a correlation between available form of organic nitrogen and antioxidant quality of tea leaves. Insignificant action of sulphur on antioxidant was observed, above 13 and below 7.00 reduces the tea antioxidant quality. Moisture level at 15% decreases the nutrients availability of tea plants. Plant age and leaf maturation also have significant correlation with antioxidant quality of tea. Maturation of tea leaf affects the accumulation pattern of antioxidant capacity was executed. Below minimum temperature and above maximum temperature, storage and development of antioxidant compounds in tea leaves may be hampered. The high variation of antioxidant activity of tea of Dooars region and soil nutrient profile of six places with different age was observed. Also this observation suggested that anti-lipid peroxidation has significant correlation with nitrogen content available in soil, which indicates that nitrogen content of soil is highly responsible for antioxidant capacity of tea. Tea industries have gone for higher input with resultant impact on chemical constituents of tea shoots responsible for quality. The major quality attributes of tea are flavour, aroma, colour, and strength. Low theaflavin content in black tea is due to over fermentation and/or long periods of storage

No industry in North Bengal produced tea from specific cultivar because while plucking shoots from tea garden, labourer never plucked shoots from individual cultivar, always mixed up and after weighing, tea shoots are moving from garden to distance factory by trolley. During transportation withering starts and it takes nearly 4hrs. Therefore, study of industrial process control *i.e*. through what process, tea industry is actually following and what the quality attribute is restored from processed intermediate to final grade, has focused through this study. For the betterment of quality tea manufacturing, withering and fermentation behaviour of selected cultivars were also studied exhaustively.

It was revealed from the study of variation of profile of UV responsive substances during industrial processing of CTC tea and the results confirmed that TLC can also be efficaciously used for rapid analysis of different tea grades and their processing intermediates during quality control like other pharmaceutical and food products for evaluation of nutraceuticals and other value-added products.

Comparative analysis of antioxidants and bioactive compounds of three basic tea products viz. CTC, orthodox and green tea were executed. From our study it can be affirmed that orthodox tea have a more potent antioxidant activity as compared to other CTC teas besides enhanced colour and aroma.

To understand more about the relationship between soil physicochemical profile phytoconstituents, antioxidant attributes and age of the plantation, PCA (Principal Component Analysis) was performed with each region of North Bengal. The twenty eight principal components were chosen on the basis of their eigenvalues, explaining the total data variance for Dooars, Terai and Darjeeling hill. In almost all cases composite soil physicochemical attributes were heavily loaded on the second principle component (PC2) and clustered as evidenced by the Dendro-hit map.

To study more about the relationship between climatic factors, profile of phytoconstituents, and antioxidant attributes, PCA was performed with each region of North Bengal. The twenty six principal components were chosen on the basis of their eigenvalues, explaining the total data variance for Dooars, Terai and Darjeeling hill. In

almost all cases climatic attribute were heavily loaded on the second principle component (PC2) and clustered as evidenced by the correlation matrix analysis.

Comparison of phytochemical constituents and antioxidant activity of organically and non-organically produced black tea were studied. For that purposes sixteen commercial tea samples of eight black tea grades, viz., orange pekoe (OP), broken orange souchans (BPS), orange fanning (OF), fanning dust (FD), flowery orange pekoe (FOP), broken orange pekoe one (BOPI), broken orange fanning (BOF), orange churamani dust (OCD) were collected from the tea gardens of Terai, Dooars and Darjeeling Hills, in a three harvesting seasons and analyzed. All the samples used in the study are the grades prepared by the different factories in North Bengal over the years. There was a wide variation in the amount of total polyphenols and antioxidants in eight different grades tested. Among the eight grades of organically produced tea, the highest phenol content and antioxidant activity was found in FOP and lowest in FD, whereas in the non-organically produced tea, highest phenol content was observed in BOPS and lowest in OF. From the results, greater amounts of polyphenol, flavonol, tannin and ortho-dihydric phenol content were found in organically produced orthodox tea, resulting in better tea quality for healthy consumption compared to the non-organically produced Black tea grades obtained from the gardens of Terai, Dooars and Darjeeling Hills.

In chapter VII, outcome of the studies has been precisely discussed.