Influence of foliar application of micronutrients on physiological characteristics and yield of Darjeeling tea (*Camellia sinensis* L.)

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Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most important beverage crops in the world. It is consumed more than any other liquid except water. Tea is a perennial plant, which repeatedly pruned at different intervals (4- 6 years in Darjeeling hill).

The major tea-growing regions of the world are South-East Asia and Eastern Africa where it is grown across a wide range of altitudes up to 2200 m a.s.l..

Productivity in tea, just like any other crop, is a function of a number of factors such as climate, soil, genotype and cultural practices. Fertilisation is one of the major determinants of yield in tea besides planting material, pruning and harvesting patterns (Drinnan, 2008). A balanced nutrition with both macronutrients and micronutrients is a requirement for tea to produce satisfactory yields.

Micronutrients, although they are required in minute quantities, play important roles in plant growth and development. They function either as catalysts or at least they are closely linked to catalytic processes within plants. Zn, B, Mn, Mo and Mg are among the essential elements for growth and development hence their deficiencies severely limit crop production.

The spraying of micronutrient mixture containing Zinc, Boron, Molybdenum, Manganese and growth promoters like tri-contanol could increase the yield level of mature tea when it became nearly stationary inspite of adequate supply of Nitrogen, Phosphate and Potash (Barua et 1969).

Tea being a vegetatively exhaustive crop, requires balanced manuring and foliar nutrition to give the maximum amount of leaf in every flush. After every flush comes a banjhi period or the resting stage where the crop gears it self up for the next phase.

During this critical period the need for foliar nutrition has come up with significance through extensive field trials and research.

Fertilisation in tea especially in Darjeeling has put more emphasis on macro elements such as N, P and K with little attention paid to fertilisation with micronutrient elements. As a result some tea estate have suffered micronutrient deficiencies, especially those of Zn and B in recent years.

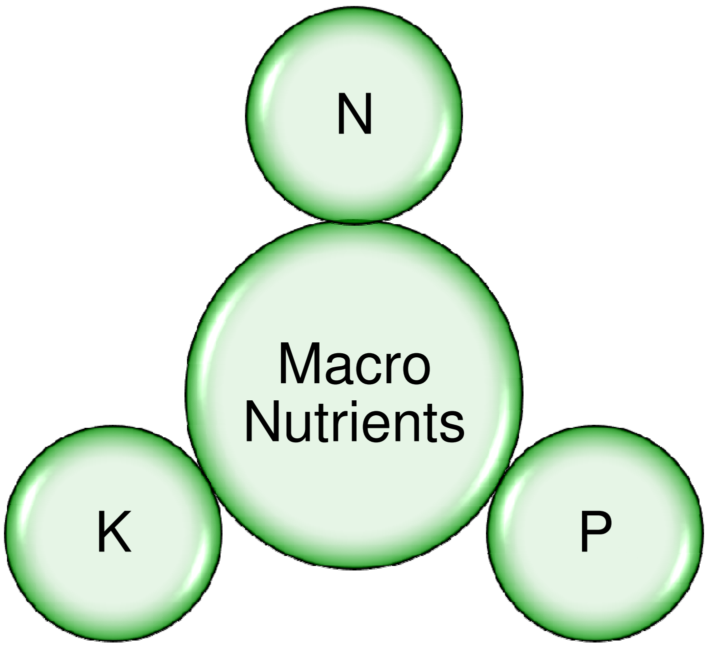
Foliar applications of Zn, Mg, B, and Mo containing fertilisers may therefore be able to improve tea yields.

This study was conducted to evaluate the effect of foliar sprays of Zn, Mg, B, Mn and Mo containing fertilisers, including two commercial micronutrient mix, on Darjeeling tea production.

Macro-nutrients

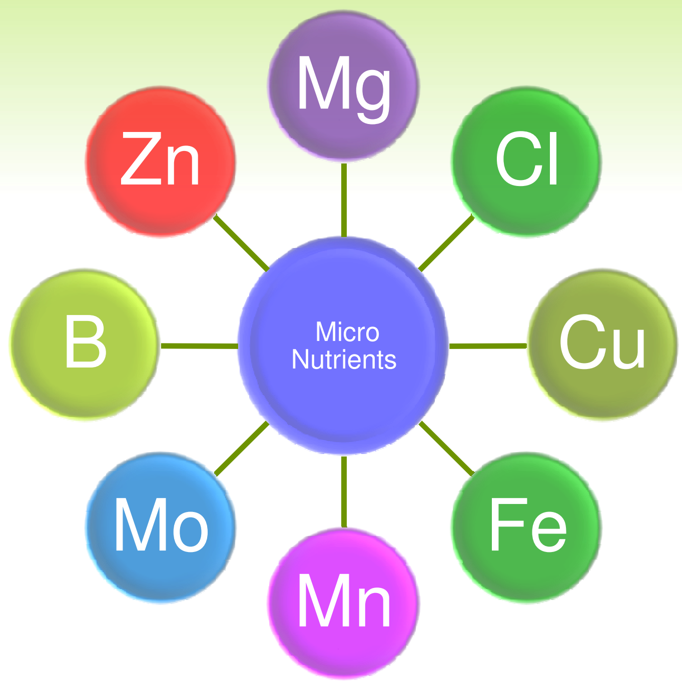
- Nitrogen promotes quantity and speed of leaf growth.
- Phosphate (P_2O_5) promotes root growth.
- Potash (K_2O), helps metabolism, imparts resistance

These nutrients are all needed for plants to grow. This may not be available in soil in sufficient quantities for optimum development and yield and hence annual application necessary.



Micro-nutrients

Promote normal health. These are main micronutrients. They include Boron (B), chloride (Cl), and copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Zinc (Zn) and including Magnesium (Mg) secondary nutrients,



Zinc (Zn) and Magnesium (Mg) promotes health and growth, foliar application in high yielding teas.

Nutrients Deficiency Symptoms in Tea plantation

N = Slow growth, fewer shoots, small leaves.

P = Dull, small leaves, weak stem, slow development.

K = Thin white stem, folded small leaves, poor recovery from prune or drought, fair central growth with poor side branches.

Ca = When pH value below 4.

Mg = When pH values below 4, also in droughty conditions.

S = Yellowing of upper leaves.

Zn = Sickle shaped leaves.

B = Resetting of new growth.

Trace Elements = Discolored leaves, curly leaf edges and stunted growth.

Role of Zinc in Tea cultivation

- Zn is an essential micronutrient, with a particular physiological function in all living systems. Its essentiality is demonstrated by its role as a co-factor in a number of enzymes in biochemical pathways that are primarily concerned with carbohydrate metabolism in photosynthesis and transformation of sugars to starch, protein metabolism, auxin growth regulators, pollen formation and maintenance of membrane integrity (Alloway, 2008).

- Zinc is involved in N metabolism of plants. Spraying of 1-2% Zn SO_4 increased nitrate reductase activity and also resulted in a 15-20% increase in N and protein content of tea shoots (Barbora et al., 1993).

- Zinc plays an important role in photosynthesis and mobilization of assimilates and has been shown to mobilize photosynthates towards pluckable shoots in tea (Barbora et al., 1993

- Leaf chlorophyll content, stomatal conductance and net photosynthesis are adversely affected by inadequate supply of Zn (Barbora et al., 1993).

Role of Mg and B in Tea Cultivation

- Magnesium is essential constituent of chlorophyll without this photosynthesis is not possible. It promotes uptake and translocation of phosphate and movement of sugar within plant.

- In case of magnesium deficiency, very distinctive interveinal chlorosis is observed with yellowing ‘V’ shape colour in lamina but veins remain green. This is common in winter months specially in Cambod origin plants.

- Boron is essential nutrient in the physiology of plants. Its roles are closely linked to the primary cell wall structure, membrane functions and reproductive growth of plants (Blevins & Lukaszewski, 1998).

- Boron is involved in the activities related to the development and strengthening of the cell wall, cell division, fruit and seed development, sugar and phosphate transport (Power & woods, 1997). Boron has been reported to be involved in the movement of Ca within plants.

Role of Mn and Mo in tea cultivation

- Manganese (Mn) is concerned with N assimilation. It has been influence on formation of chloroplast, chlorophyll and photosynthesis. In Mn deficient plant there is more accumulation of soluble photosynthates.

- In Mn deficient plants there is more accumulation of soluble organic nitrogenous compounds-free amino acids, amides and a decrease in organic nitrogenous compounds-free amino acids, amides and a decrease in protein content. It increases protein content in tea shoot.

- Molybdenum (Mo) is a constituent nitrate reductase and associated with Nitrogen utilization and nitrogen fixation. Mo deficiency results in decreased concentration of sugar. It has a role in carbohydrate metabolism.

- The plant with molybdenum deficiency show intervened chlorosis and mottling of lower leaves, followed by marginal necrosis and in-folding.

- Sometimes normal leaves get twisted and elongated with lamina showing various degrees of narrowness and irregularities. Lamina becomes cupped and abnormally dark green. The veins may show purple colouration.

Objectives

- To study was conducted in order to evaluate the effect of different formulations of foliar applied Zn, Mg, B, Mn and Mo on physiological characteristics of tea plants.

- Specifically, the study aimed at evaluating the effects of foliar sprays of nutrients (Pure Salt), including two commercial micronutrient mixes, on productivity of Darjeeling tea.

Materials & Methods

Experimental site and plant material

- The study was conducted at the experimental farm of Darjeeling Tea Research & Development Centre, Kurseong (26.9^{0}N, 88^{0}12 E, altitude 1347 m).

- The topography comprised of moderate slopes (25-30%).The topsoil is about 45 cm in depth and the sub soil is stony. The soil was an Umbric Dystrochrept, moderately permeable and moderately well drained.

- The soil texture is sandy loam.

- Used Old China Cultivation

- Randomized Block Design and Replication three (3)
- Each plot consists of 50 plants.
- The plants were not irrigated as this is the general practice in this region due to unavailability of irrigation facility.
- The pure salt mixtures were prepared in the laboratory with laboratory grade chemicals viz., $ZnSO_4$, $MgSO_4$, $MnSO_4$, H_3BO_4 and $(NH_4)_6MO_7O_{24}$ and two commercial grade micronutrient formulations viz. Micromix-5 and Trasco-5 (Tea special) were used.
- A common dose of N: P: K:: 90:45:90 kg/ha through Urea, Rock phosphate and MOP was applied respectively.

Treatments

T1	=	Control
T2	=	2% Zn (10 kg/ha $ZnSO_4.7H_2O$)
T3	=	1% B (5 kg/ha of H_3BO_4)
T4	=	2% Zn + 2% Mg (10 kg/ha $ZnSO_4.7H_2O$ + 10 kg/ha $MgSO_4$)
T5	=	2% Zn + 1% B + 0.5% Mo (10 kg/ha $ZnSO_4.7H_2O$, 5 kg/ha of H_3BO_4 and 2.5 kg/ha $(NH_4)_6$ $MO_7O_{24}.4H_2O$)
T6	=	2% Zn + 2% Mg + 1% B (10 kg/ha $ZnSO_4.7H_2O$ + 10 kg/ha $MgSO_4$ + 5 kg/ha H_3BO_4)
T7	=	2% Zn + 1% Mn + 1% B (10 kg/ha $ZnSO_4.7H_2O$ + 5kg/ha $MnSO_4$ + 5 kg/ha H_3BO_4)
T8	=	Micromix-5 (Zn 5.3%, B 1.0%, Mo 0.1%, Mn 5.0%, Cu 2.4%) – 1.5kg/ha
T9	=	Trasco-5 (Tea Special- Mg 2.0%, S 8.5%, Zn 9.0%, Mn 3.0%, B 1.0%, Mo 0.5%)- 400ml/ha.

Selection of chemicals /inputs

Selection of chemicals was based on their solubility in water considering the fact that the chemicals were to be applied as foliar spray applications.

Boric acid is highly soluble in water and is more suitable for foliar application than other forms of B such as borax and borates (Zekri & Obreza, 2009).

Zinc sulphate and Magnesium sulphate were soluble in water and hence suitable for foliar application (Fageria et,al., 2009).

Manganese sulphate and Ammonium Molybdenum were included because it is soluble in water also.

Selected Commercial Products

Trasco – 5 (Tea Special):

- It is a combined mixture of micronutrient formulation made specifically for tea crop for foliar application.
- The application of Trasco -5 (Tea Special) increase the vegetative growth of leaves, increases shoot weight, increases chlorophyll and there by enhance yield.

MICROMIX – 5:

- Micromix-5 is a balanced fertilizer micronutrients mixture for healthy growth, higher yield, and better quality of tea , fruit and vegetables.
- The composition of Micronutrient Fertilizer-Grade-5 (MICROMIX-5) is rich in Cu (2.4%), Mn (5.0%), Zn (5.3%), B (1.0%), and Mo (0.1%). Non-phytotoxic when used as recommended doses 2 to 5 gm. / litre of water. (Perennial plantation crops – 2spray)

Methods and Timing of Spraying

Application of foliar sprays was done using a 12.5 litre Knapsack sprayer, which was calibrated according to procedures described by Rattan (1988) in order to ensure that the exact dosage was applied and that the chemicals were evenly distributed onto the tea plants.

Spraying was done in the morning twice a year during the month in March/April and August/September every year.

Days on which spraying was done coincided with the plucking days in such a way that spraying immediately followed plucking and the next plucking was done after 10 days.

In the first application, the plan was to spray on the plucking day soon after plucking during the month of March. Care was taken not to spray on rainy days

Some important information's to the application of fertilizers

Q1. Is it helpful to fertilize right after pruning of tea bush?

Ans. - No, for two reasons:

- The plant doesn't have enough leaves to evaporate water and pull water up the xylem tubes to move the fertilizer from the roots into the stems and leaves.
- Because there are no leaves to produce sugar, the roots do not have enough energy to absorb fertilizer from the soil. Absorption of fertilizer is not like a sponge absorbing water; instead, it is a complicated process that takes energy. So: fertilize a month before, or a month after, pruning.

Q2. Is it a good idea to apply foliar fertilizers when the sun is hot?

Ans.- No, for two reasons:

- If the sun is hot, it will evaporate some of the spray from your backpack sprayer even before it reaches the leaf.
- While the sun is hot, the leaves will have their stomata closed. Foliar fertilizers are absorbed through the stomata. But if stomata are closed, they cannot enter the plant, and so they will just evaporate on the outside of the leaf. So: apply foliar fertilizers in the cool late afternoon.

Environmental parameters during the study period (Average mean of five years during 2008 to 2013).

The experimental period was characterized by a moderate temperature during spring time (20.71 ^{0}C), a high temperature average from May to September (23.2 ^{0}C) and an average of 15.7 ^{0}C during December to February period.

- The maximum rainfall on an average of 1057.3 mm was recorded at middle of rainy season (July).
- The ambient temperature (22 to 24 ^{0}C), relative humidity (88 to 91%) were high during rains

Month's	Air Temperature		Total Rainfall	Mean Relative Humidity
	Max. (^{0}C)	Min. (^{0}C)	mm	(%)
April	20.71	12.32	149.3	81.1
May	22.03	13.68	290.4	86.0
June	23.73	16.52	806.6	91.0
July	24.00	16.86	1057.3	89.5
August	23.42	15.07	958.0	90.1
September	22.56	14.38	538.0	89.5
October	20.93	14.90	54.7	86.5
November	18.16	9.73	0.0	85.0
December	16.80	8.90	2.1	79.0
January	13.81	4.39	2.9	85.5
February	16.45	6.38	4.2	81.2
March	19.28	9.70	6.8	71.0

Measurement techniques

- Harvesting was done by hand plucking of tender shoots from the tea bushes on every 7 days intervals during the study. In Darjeeling, yield recording started from 2nd week of March and ended the last week of November (Twenty-six cycles per year). December to February no harvesting of green leaf.

- On each plucking day, mass of plucked leaf from each plot was recorded and expressed as made tea yield by multiplying the green leaf yield by factor of 0.22.

- Shoot density was recorded weekly by using a 0.50 X 0.50 m square quadrate, which was thrown randomly on the plucking table at three positions in each plot. Different shoot categories were counted at each position and the number of shoots per m-2 was calculated. This was performed once a month.

- The leaf sample was separated into different categories of shoots from the 100gm sample of green leaf from the plucked leaf once a month, which were: 1L+b, 2L+b, 3L+b and Banjhi shoots.

- During 2008 to 2013, P_N, g_s, *E and WUE* were monitored three times in a month at the beginning, middle and end of April, July, October and January, using a portable photosynthetic system (Li 6200, Li -Cor, Nebraska, USA) with a well mixed 390 cm^3 chamber as described (Li-Cor Inc., 1987).

- All measurements were made between 10 00 and 12 00 hours when the maximum values of P_N .

- Chlorophyll content of freshly harvested leaves collected from the opposite branches to those for P_N measurement was estimated .

Results and Discussion

Net photosynthetic rate (μ mol $m^{-2}s^{-1}$)

- Maximum photosynthetic rate (P_N 12.27 μ mol $m^{-2}s^{-1}$) was observed with the application of 2% Zn + 2% Mg + 1% B (T6) followed by 2% Zn + 1% Mn + 1% B (11.33 μ mol $m^{-2}s^{-1}$) and minimum in control (T1). But the lowest Pn was observed with the application of 400ml/haTrasco-5 (Tea Special)(9.03 μ mol $m^{-2}s^{-1}$).
- The maximum photosynthetic active radiations were observed during the summer season in coincidence with high temperature.
- Pn was higher when photosynthetic photon flux density (PPFD) increased from 1000 to 1400 μ mol $m^{-2}s^{-1}$ (autumn). Lowest PPFD was in rainy season which affected the Pn.
- Zinc is involved in N metabolism of plants. Spraying of 1-2% Zn SO_4 increased nitrate reductase activity and also resulted in a 15-20% increase in N and protein content of tea shoots (Barbora et al., 1993).

Micronutrients and doses		Pn (μ mol $m^{-2}s^{-1}$)
T1	Control (No spray)	8.89
T2	2% Zinc	10.58
T3	1% Boron	9.37
T4	2% Zinc + 2% Magnesium	10.49
T5	2% Zinc + 1% Boron + 0.5% Molybdenum	11.00
T6	2% Zn + 2% Mg + 1% B	12.27
T7	2% Zn + 1% Mn + 1% B	11.33
	Micromix-5 (1.5 kg/ha)	9.18
T9	Trasco-5 (Tea special) - 400ml/ha	9.03
Sem		0.27
CD at 5%		0.82

Stomatal Conductance **(mol $m^{-2}s^{-1}$)**

- The maximum value of g_s 0.32 mol $m^{-2}s^{-1}$ was recorded with the application of 2% Zn + 2% Mg + 1% B and minimum in control (T1) but lowest was notice inTrasc-5 (Tea special) 0.22 mol $m^{-2}s^{-1}$.
- Higher value of gs was recorded in rainy season and lowest in summer. In general, the decrease in g_s was more pronounced in moisture stress period.

- Stomatal conductance is a key internal factor affecting Pn of tea. Because of the sensitivity of stomatal opening to several stimuli form the external environment (i.e. light intensity, water availability, leaf temperature, VPD etc.), very often, gs mediates the response of Pn to external factor as well.
- Generally, there is a positive relationship between Pn and gs because increased stomatal opening (i.e. higher gs) allow flux of CO2 for photosynthesis.
- Smith et al. (1994) also observed a similar positive correlation between Pn and gs in as much as gs was below 30 m mol H2O $m^{-2}s^{-1}$.

	Micronutrients and doses	gs (mol $m^{-2}s^{-1}$)
T1	Control (No spray)	0.21
T2	2% Zinc	0.25
T3	1% Boron	0.30
T4	2% Zinc + 2% Magnesium	0.29
T5	2% Zinc + 1% Boron + 0.5% Molybdenum	0.30
T6	2% Zn + 2% Mg + 1% B	0.32
T7	2% Zn + 1% Mn + 1% B	0.30
T8	Micromix-5 (1.5 kg/ha)	0.25
T9	Trasco-5 (Tea special) - 400ml/ha	0.22
Sem		0.01
CD at 5%		0.03

Transpiration (*E*-m mol $m^{-2}s^{-1}$

- Higher value of E (4.01 m molm^{-2}s^{-1}) notice in T4 and lowest in Micromix-5. T6 showed lower rate of transpiration than other treatments. Transpiration rate was lowest in summer season and higher in rain, though the PPFD reached minimum but the temperature, Sm (Soil moisture), wind velocity and RH were reasonably high.
- Barbora, (1994) also reported a sharp decline of *E* with reduced soil moisture in Assam tea plantation.
- Water use of tea and its controlling factors have been studied extensively (Stephens and Carr, 1991; Kigalu,2007; Anandacoomaraswamy et at., 2000). However, water use (evapotranspiration) include both transpiration form the foliage canopy and soil evaporation.
- A well-maintained tea canopy (including nutrients) covers the ground almost completely allowing very little solar radiation to penetrate down to the soil surface

	Micronutrients and Doses	E - (m mol $m^{-2}s^{-1}$)
T1	Control (No spray)	3.87
T2	2% Zinc	3.84
T3	1% Boron	3.90
T4	2% Zinc + 2% Magnesium	4.01
T5	2% Zinc + 1% Boron + 0.5% Molybdenum	3.91
T6	2% Zn + 2% Mg + 1% B	3.59
T7	2% Zn + 1% Mn + 1% B	3.64
T8	Micromix-5 (1.5 kg/ha)	3.43
T9	Trasco-5 (Tea special) - 400ml/ha	3.46
Sem		0.05
CD at 5%		0.14

Water Use Efficiency (μ mol/ $mmol^{-1}$)

- **Maximum WUE was recorded in T6 (3.41 μ mol $mmol^{-1}$) and minimum in control T1 (2.29 μ mol m mol^{-1}).** In different treatments, maximum WUE was associated with relatively lower *E* and lower g_s. Maximum WUE was recorded in winter (4.09 μ mol/ $mmol^{-1}$) and minimum in rainy season (1.79 μ mol $mmol^{-1}$).

- A positive correlation existed between WUE and P_N which is in conformity with the findings of Ghosh Hajra and Kumar (2002).

Average mean of five years during 2008 to 2013. WUE is the amount of yield produced per unit of water used through evapotranspiration. It could be affected by factors influencing shoot growth and water use. At lower Temperature (cool dry season), WUE respond more to nitrogen fertilizer than to rainy.

Stephens and Carr (1991) showed that WUE of tea is influenced by water availability, nitrogen application and season.

	Micronutrients and Doses	WUE- (μ mol/ $mmol^{-1}$)
T1	Control (No spray)	2.29
T2	2% Zinc	2.76
T3	1% Boron	2.40
T4	2% Zinc + 2% Magnesium	2.61
T5	2% Zinc + 1% Boron + 0.5% Molybdenum	2.89
T6	2% Zn + 2% Mg + 1% B	3.41
T7	2% Zn + 1% Mn + 1% B	3.10
T8	Micromix-5 (1.5 kg/ha)	2.71
T9	Trasco-5 (Tea special) - 400ml/ha	2.60
Sem		0.07
CD at 5%		0.21

Effect of foliar application of micronutrients (Pure salt) and Commercial product on chlorophyll content

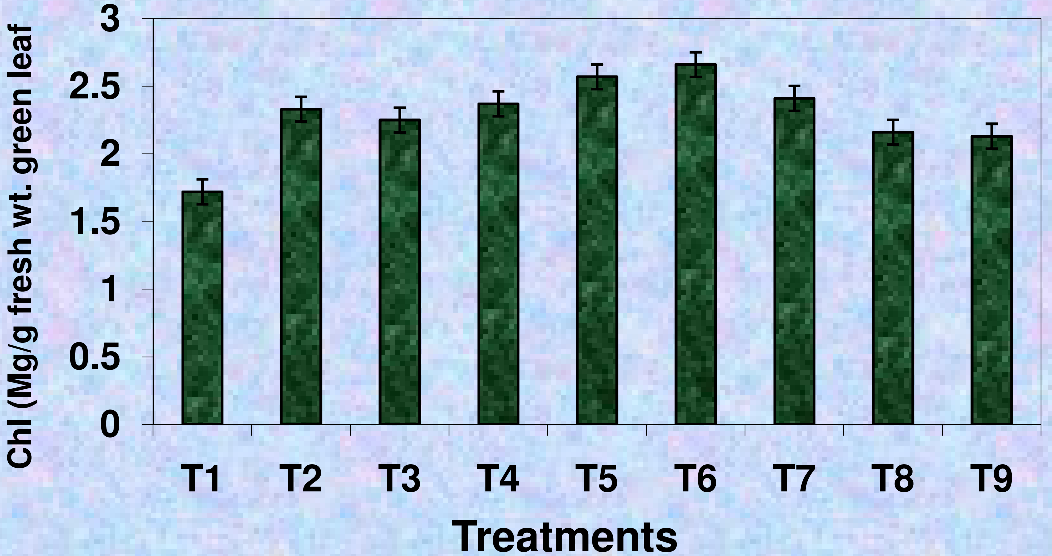
❖ Average mean of five year during 2008 to 2013. Vertical bar indicate standard error of means.

❖ The chlorophyll content is the most important plant pigment playing a vital role in determining the photosynthetic efficiency and productivity of the plant.

❖ The treatment comprised of 2% Zn + 2% Mg + 1% B (T6) was found effective to enhance the chlorophyll pigments followed by 2% Zn + 1% B + 0.5% Mo (T5) and 2% Zn + 1% Mn + 1% B (T7) in photosynthetic pigments over the control.

❖ Leaf chlorophyll content, stomatal conductance and net photosynthesis are adversely affected by inadequate supply of Zn (Barbora et al., 1993).

❖ Magnesium (Mg) is essential constituent of chlorophyll without this photosynthesis is not possible.



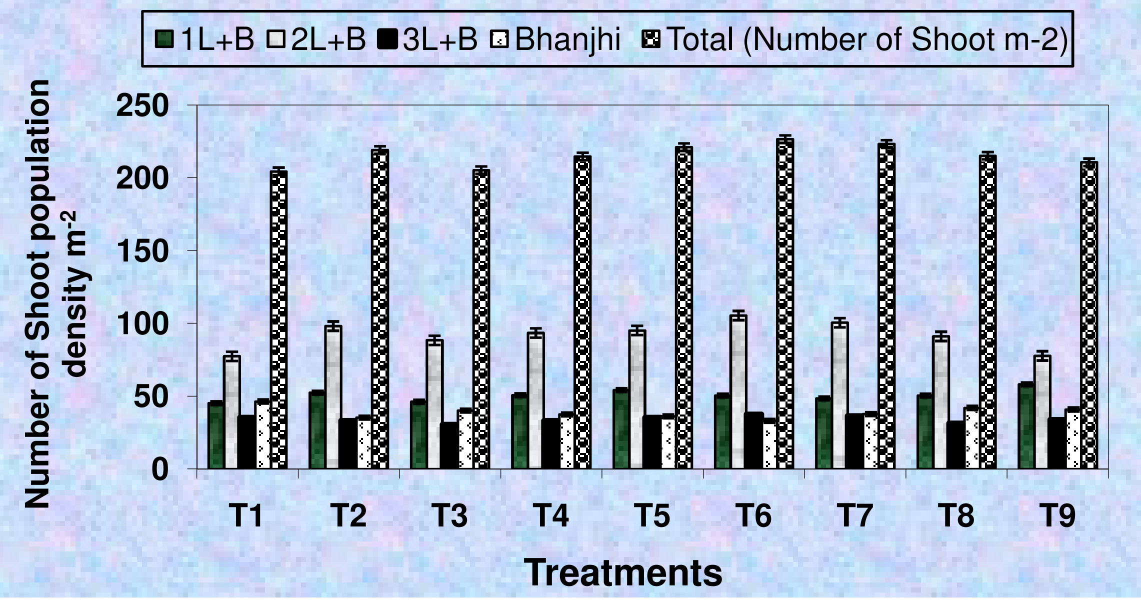
Effect of foliar doses of micronutrients on shoot population density of Darjeeling tea

- A collection of individual shoots containing two leaves and a bud (2L+b) or three leaves and a bud (3L+b) comprise the economic yield of the tea plant (Mathews & Stephens 1998).

- A significant increase in shoot density of 2L+b, 3L+b and total number of shoots was observed where Zinc sulphate with Boric acid and Magnesium sulphate was applied.

- Zinc sulphate, Magnesium sulphate and Boric acid mixture tended to give the highest number of 2L+b, 3L+b shoots m^{-2} and total number of shoots m^{-2} . But mixture of Zn + Mg + B was reduced the banjhi/dormant shoots m^{-2} .

- Field observations have shown that the density of dormant shoots are less during periods of higher leaf yield and high during periods of lower leaf yield (Wijeratne, 1994).
- Average weekly mean data of five year during 2008 to 2013. Vertical bar indicate standard error of means.



Different plucking shoots

After producing the fish leaf, the terminal bud produces normal flush leaves.

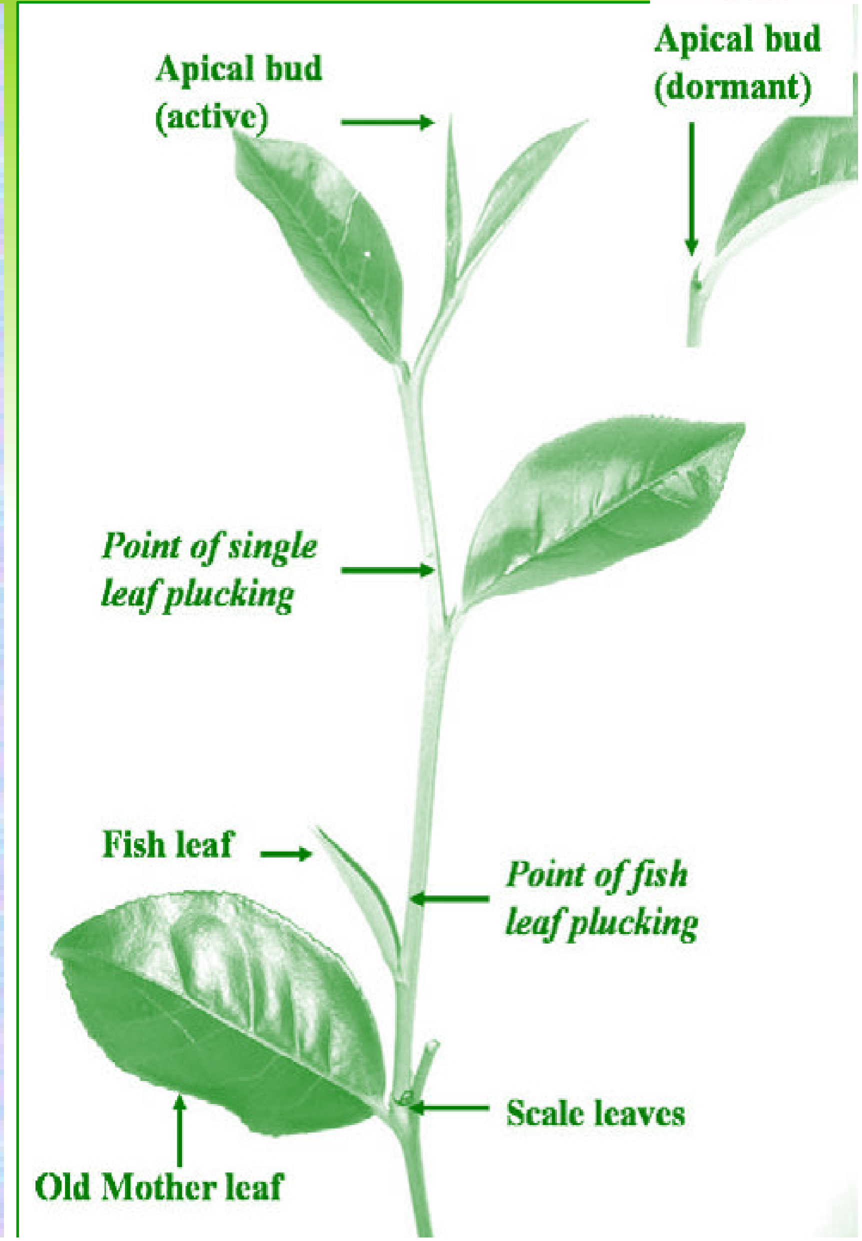
1L+ b (one leaves and a bud)

2L+ b (Two leaves and a bud)

3L+ b (Three leaves and a bud)

The dormant bud *banjhi* is a few millimeters long tiny bud usually covered by leaf hairs. It could be easily differentiated from an active terminal bud as the former is very small compared to the latter.

Foliar applications of Zn, Mg and B containing fertilizers may therefore be able to improve tea production.



Yield of processed tea

- In the present study, The average annual yield of made tea was recorded only 550 kg/ha .
- It is observed that T6 treatment viz., pure salt of Zinc, Magnesium and boron recorded the highest yield viz., 626.05 kg/ha of made tea and also the same treatment recorded the highest yield in the 5 individual years.
- The made tea yield was significantly affected by the treatments at Kurseong.
- The percent increase of yield is also 19.04 % more than control (T1) which did not receive any micro-nutrient. Hence, it is inferred that the efficacy of the pure salts is remarkably higher in increasing yield than the commercial products probably because of the less impurities in them and greater assimilation of the nutrients.
- Average mean of five years during 2008 to 2013.
- Hence, it is inferred that the efficacy of the pure salts is remarkably higher in increasing yield than the commercial products probably because of the less impurities in them and greater assimilation of the nutrients.

Treats.	Name of micronutrients and doses	Yield of made tea (Kg/ha)	%
T1	Control (No spray)	525.90	---
T2	2% Zinc	562.90	7.03
T3	1% Boron	553.63	5.27
T4	2% Zinc + 2% Magnesium	596.53	13.43
T5	2% Zinc + 1% Boron + 0.5% Molybdenum	609.73	15.94
T6	**2% Zn + 2% Mg + 1% B**	**626.05**	**19.04**
T7	2% Zn + 1% Mn + 1% B	611.63	16.30
T8	Micromix-5 (1.5 kg/ha)	583.17	10.89
T9	Trasco- 5 (Tea special)- 400ml/ha	578.17	9.94

Sem = 7.26 CD at 5% = 22.02

Conclusion

- Foliar applications of Zn, Mg, B and Mn containing fertilizers may therefore be able to improve tea yields.

- The micronutrients effects were significant on the yield. Based on results Zinc sulphate, magnesium sulphate and boric acid was the best found over all the treatment and recorded increment in yield 19.04% over control.

- It is also observed that foliar application of micronutrients increased the primary metabolites like photosynthetic pigments.

- Magnesium is the only mineral constituent in the chlorophyll molecule that regulates photosynthesis.

- it is inferred that the efficacy of the pure salts is remarkably higher in increasing yield than the commercial products probably because of these less impurities in them and greater assimilation of the nutrients.

चाय पियो मस्त जियो !
Chai piyo mast jiyo !

Thank you