RESPONSE OF FOLIAR APPLICATION OF MICRONUTRIENTS ON PHYSIOLOGICAL, BIOLOGICAL CHARACTERISTICS AND YIELD OF TEA (Camellia sinensis L.) IN DARJEELING HILL

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Abstract
A study was conducted at experimental Farm, Darjeeling Tea Research and Development Centre, Kurseong, Darjeeling during the year 2009 to 2015 to evaluate the response of different micronutrients applied as foliar application on yield and physiological characteristics of mature tea. Micronutrients, although they are required in meagre quantities, play important roles in plant growth and development. Current fertilizer recommendation have emphasized much on macronutrients, such as N, P and K, but little attention has been paid to micronutrient elements despite continuous removal through harvesting. The uses of micronutrients have emerged as an important tool to enhance the productivity. The application of micronutrient was done twice in a year during March-April and August-September. The recommended dose of NPK was common for all treatment and applied at 90:45:90 Kg NPK/ha through Urea, RP and MOP. Highest net photosynthetic rate ($P_N$ 12.27 μmol m$^{-2}$s$^{-1}$) was observed from the plot where foliar application of 2% Zn + 2% Mg + 1% B (10 kg/ha ZnSO$_4$.7H$_2$O + 10 kg/ha MgSO$_4$ + 5 kg/ha H$_3$BO$_4$) was done which was closely followed by 2% Zn + 1% Mn + 1% B (11.33 μmol m$^{-2}$s$^{-1}$) while it was lowest in the plot where Trasco-5 (Tea Special) @ 400ml/ha was applied (9.03 μmol m$^{-2}$s$^{-1}$). Maximum Chlorophyll content was recorded from the plot where 2% Zn + 2% Mg + 1% B applied while it was lowest in control plot. Highest water use efficiency (WUE) was recorded from the plot where 2% Zn + 2% Mg + 1% B applied than other treatment. Positive correlation was observed between $P_N$ and WUE. Among pure salts Zinc, Magnesium and Boron (10 kg/ha ZnSO$_4$.7H$_2$O + 10 kg/ha MgSO$_4$ + 5 kg/ha H$_3$BO$_4$) gave the highest made tea yield (626.05 kg/ha), the increment of yield was 19.04 % over the control plot. The physiological analysis of photosynthetic pigments like chlorophyll revealed that there was positive and significant increase in all above parameters in treated plots as compared to control plot.

Key words: Camellia sinensis, fertilizers, Micronutrients, Photosynthesis, Chlorophyll, made tea yield.

I. INTRODUCTION

Tea is favourite beverage of people of India and all over 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 shoots are plucked at regular intervals (6-13 days) and removed a considerable amount of various elements from the soil-plant system. These nutrients have to be supplemented through fertilizer application. The current fertilizer recommendation in tea, especially in Darjeeling, emphasis the application of major nutrients, particularly N, P and K, whilst micronutrients are not regularly applied in tea bushes, hence their removal from the soil continues without an organized replacement plan. Tea yield increases sharply with increased of N and K to a certain point [6]. 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 [7]. There are numerous commercial micronutrient products available for use by the growers. These range from single elemental products to multi-micronutrient mixtures. These multi-micronutrient mixtures have the potential to serve as a remedy to micronutrient deficiencies, especially in cases where multiple micronutrient deficiencies occur.

Zinc is an essential micronutrient, with a particular physiological function in all living systems. Its essentiality is demonstrated by its role as a cofactor 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 [2]. Zinc is involved in N metabolism of plants. Spraying of 1-2% Zn SO₄ increased nitrate reductase activity and also resulted in a 15-20% increase in N and protein content of tea shoots [5]. Zinc is required for the synthesis of IAA, which is responsible for active shoot growth in tea [1], [21]. Zinc plays an important role in photosynthesis and mobilization of assimilates and has been shown to mobilize photosynthates towards pluckable shoots in tea [5]. Approximately 9.0% of assimilates were partitioned towards pluckable shoots in tea plants treated with 1% ZnSO₄ compared to 0.4% in untreated plants. Leaf chlorophyll content, stomatal conductance and net photosynthesis are adversely affected by inadequate supply of Zn [5].

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 [9]. 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. Boron has been reported to be involved in the movement of Ca within plants.

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 motting 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.

Trasco -5 (Tea Special) 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.

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 itself 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.
Keeping above fact in view, a study was therefore conducted in order to evaluate the effect of different formulations of foliar applied Zn, Mg, B, Mn and MO on yield and physiological characteristics of tea plants. Specifically, the study aimed at evaluating the effects of foliar sprays, including two commercial micronutrient mixes, on productivity of Darjeeling tea. It also aimed at establishing the effect of foliar sprays on concentrations of Zn, Mg, B and other nutrient elements in the leaves of treated tea bushes. It was hypothesized that foliar applied fertilizers containing Zn, Mg and B would have significant effects on the yield.

II. MATERIALS AND METHODS

Experimental site and plant material

The study was conducted at the experimental farm of Darjeeling Tea Research & Development Centre, Kurseong (26.9°N, 88°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. Infiltration rate was 4 -6 cm h⁻¹ measured by water hydrograph method in the field (unsaturated) conditions. The soil texture is sandy loam. The minimum (base) air temperature for shoot extension and development varies from clone to clone in the range of 8-15 °C. Above the base temperature, the rates of extension and development are linear up to a maximum of about 30- 35 °C. Unlike extension growth, photosynthesis does not stop at 12 °C. In Darjeeling, photosynthesis was observed to continue during winter (higher rate than summer and rains) when minimum temperature fall below 12 °C and the tea bushes were dormant. In Darjeeling, higher rate of photosynthesis was recorded in cold and only 15 % depression in comparison to autumn was observed [13]. Although shoot growth diminishes as the temperature falls and ceases below 12°C but photosynthesis accompanied by slow rate of respiration continues even at lower temperature. As a result, the bush gains in weight in a cool climate without concomitant increase in shoot weight.

The plots prepared were laid out in a randomized block design with three replications: T1 = Control, T2 = 2% Zn (10 kg/ha ZnSO₄.7H₂O); T3 = 1% B (5 kg/ha of H₃BO₃); T4 = 2% Zn + 2% Mg (10 kg/ha ZnSO₄.7H₂O + 10 kg/ha MgSO₄); T5 = 2% Zn + 1% B + 0.5% Mo (10 kg/ha ZnSO₄.7H₂O, 5 kg/ha of H₃BO₃ and 2.5 kg/ha (NH₄)₆ MO₇O₂₄.4H₂O); T6 = 2% Zn + 2% Mg + 1% B (10 kg/ha ZnSO₄.7H₂O + 10 kg/ha MgSO₄ + 5 kg/ha H₃BO₃); T7 = 2% Zn + 1% Mn + 1% B (10 kg/ha ZnSO₄.7H₂O + 5 kg/ha MnSO₄ + 5 kg/ha H₃BO₃); T8 = Micromix-5 (Zn 5.3%, B 1.0%, Mo 0.1%, Mn 5.0%, Cu 2.4%) – 1.5kg/ha and 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. Spacing for planting was 90 cm x 60 cm x 60 cm and the distance from hedge to hedge was 90 cm, row to row 60 cm, and plant to plant 60 cm. 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., ZnSO₄, MgSO₄, MnSO₄, H₃BO₄ and (NH₄)₆MO₇O₂₄ and two commercial grade micronutrient formulations viz. Micromix-5 and Trasco-5 (Tea special) were used. The dose of the spray was the conventional ones and the one prescribed by the respective companies for the commercial products. Spraying was done twice during March-April and August-September. A common dose of N: P: K:: 90:45:90 kg/ha through Urea, Rock phosphate and MOP was applied respectively.

Measurement techniques:

During 2009 to 2015, Pₜ, gₛ, and E 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 cm³ chamber as described [17]. This portable instrument has internal programmed to calculate physiological quantities from measurements of air and leaf temperatures, humidity and CO₂ concentrations. Assimilation rates are computed in this instrument by
assuming linear rates of change in water vapour and CO₂ concentrations within the leaf chamber. All data points during a measurement period were fitted using linear regression techniques. The humidity within the chamber was kept constant during the measurement period in order to get satisfactory results as observed by [15]. Dark-green healthy looking mature leaves at the surface of the canopy and fully exposed to incident sunlight were used for the observations. Such leaves are often referred to as ‘maintenance’ foliage. Three plants randomly selected from each replicated plot were assessed on every recording (972 reading). Efforts were made to ensure that measurements were taken only when there was no cloud cover. All measurements were made between 10 00 and 12 00 hours when the maximum values of $P_N$ and other physiological parameters were recorded in the diurnal study [12]. The water use efficiency (WUE) was calculated as the ratio of CO₂ assimilated to water transpired. Leaves were not brought into horizontal position during the measurement to avoid sudden change in incident quantum flux. The infrared gas analyzer had been recalibrated using compressed CO₂ gas immediately before the experimental work.

At monthly intervals, Chlorophyll content of freshly harvested leaves collected from the opposite branches to those for $P_N$ measurement was estimated according to the method described by [4], after extraction with 80% acetone in the dark and using the Hitachi (U 2000) double beam spectrophotometer.

Shoot density was recorded weekly by using a 0.50 X 0.50 m square quadrat, 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. Recording started in June 2008 and ceased in November 2015 when shoot numbers reached a peak. Shoots of harvestable size were plucked and counted. Shoots (two leaves and a terminal bud) were harvested at weekly intervals between March and October (Twenty-six cycles per year) from all the plots. Harvesting was carried out throughout the season by the same pluckers. The total fresh mass of the shoots from each plot was weighed at each harvest and converted to the made tea equivalent using a constant value of 0.22 [3]. In the Darjeeling Hills, flushing of the tea crop starts at the end of March and after a sequence of production of normal leaves in April the shoot goes dormant for a short period during May. Thereafter, harvesting of the tea crop continues until September, declines considerably towards the end of October and then ceases during November until flushing starts again at the end of March.

III. RESULTS AND DISCUSSION

Productivity in tea, just like any other crop, is a function of a number of factors such as climate, soil, genotype and cultural practices. Fertilization is one of the major determinants of yield in tea besides planting material, pruning and harvesting patterns [10]. A balanced nutrition with both macronutrients and micronutrients is a requirement for tea to produce satisfactory yield and products of desired quality. 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, Cu, B and Mn are among the essential elements for growth and development hence their deficiencies severely limit crop production.

The rainfall pattern in during study period was characterized by a scant rain in winter and summer (from November to March) and maximum in Rainy season (from June to August). The maximum rainfall on an average of 1057.3 mm was recorded at middle of rainy season (July). The experimental period was characterized by a moderate temperature during spring time (20.71 °C), a high temperature average from May to September (23.2 °C) and an average of 15.7 °C during December to February period. In Darjeeling, the extension growth stops at monthly mean maximum and minimum temperature of 18 °C and 10 °C respectively in November and it starts flushing at the middle of March when maximum and minimum temperature exceed 20 °C and 12 °C respectively. However, the ambient temperature (22 to 24 °C), relative humidity (94 to 96%) and soil moisture were high during rains. [14] Reported that relative
Table 1. Environmental parameters of the experimental site during the study period (Average mean of five years during 2009 to 2015)

| Months | Air Temperature | Total Rainfall | Mean Relative Humidity |
|--------|----------------|----------------|------------------------|
|        | Max. (0C)      | Min. (0C)      | mm                     | (%)        |
| April  | 20.71          | 12.32          | 149.3                  | 81.1       |
| May    | 22.03          | 13.68          | 290.4                  | 86.0       |
| Jun    | 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       |
| Sept   | 22.56          | 14.38          | 538.0                  | 89.5       |
| Oct    | 20.93          | 14.90          | 54.7                   | 86.5       |
| Nov    | 18.16          | 9.73           | 0.0                    | 85.0       |
| Dec    | 16.80          | 8.90           | 2.1                    | 79.0       |
| Jan    | 13.81          | 4.39           | 2.9                    | 85.5       |
| Feb    | 16.45          | 6.38           | 4.2                    | 81.2       |
| March  | 19.28          | 9.70           | 6.8                    | 71.0       |

Air humidity 80-90% is favourable for growth of tea plant but shoot growth is inhibited and adversely affected if it is below 50% and 40% respectively. The maximum photosynthetic active radiations were observed during the summer season in coincidence with high temperature. Table 1 summarizes the most important environmental parameters recorded during the experimental period.

Foliar applications of Zn, Mg, B and Mn containing fertilizers may therefore be able to improve tea yields. This study was conducted to evaluate the effects of foliar sprays of Zn, B, Mn, Mo and Mg containing fertilizers, including two commercial micronutrient mixtures, on yield and physiological characteristics of tea. Results have shown that made tea yield was significantly affected by the treatments at Kurseong. The results revealed that 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 (Table 2). Magnesium is the only mineral constituent in the chlorophyll molecule that regulates photosynthesis. In addition, it acts as an activator of many enzyme systems; also zinc is necessary for the synthesis of indole acetic acid (IAA), which is responsible for active shoot growth [22] & [23].

Table 2. Effect of foliar application of various micronutrients (Pure salt) and commercial production on Yield of processed tea (Average mean of five years during 2009 to 2015).

| Treatments | 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 |
The rainfall pattern in during study period was characterized by a scant rain in winter and summer (from November to March) and maximum in Rainy season (from June to August). The maximum rainfall on an average of 1057.3 mm was recorded at middle of rainy season (July). The experimental period was characterized by a moderate temperature during spring time (20.71 °C), a high temperature average from May to September (23.2 °C) and an average of 15.7 °C during December to February period. In Darjeeling, the extension growth stops at monthly mean maximum and minimum temperature of 18 °C and 10 °C respectively in November and it starts flushing at the middle of March when maximum and minimum temperature exceed 20 °C and 12 °C respectively. However, the ambient temperature (22 to 24 °C), relative humidity (94 to 96%) and soil moisture were high during rains. [14] Reported that relative

Effects of Zn on tea yields and quality have been reported by several authors [8], [18], [19] & [11]. [8] indicated a 20% yield increase in tea due to application of Zn alone, but when it was applied in combination with other element such as B, Mn, and Mo no further yield increase was observed and reduction in yield relative to control for some combinations occurred.

The maximum shoot population density recorded in July probably accurately reflected the total number of shoots of all sizes present at that time. The small shoot number following this and subsequent peaks occurred because enough large shoots to be included in the count had already been harvested whilst their replacements had not yet developed their first leaf. A collection of individual shoots containing two leaves and a bud (2L+b) or thee leaves and a bud (3L+b) comprise the economic yield of tea plant [20]. Results on shoot density for 1L+b, 2L+b, 3L+b and dormant (banjhi) shoots and total number of shoots m$^{-2}$ are presented in figure 1.

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+, 3L+b shoots m$^{-2}$ and total

| Treatments | Number of Shoot population density m$^{-2}$ |
|------------|------------------------------------------|
| T1         | 100                                      |
| T2         | 150                                      |
| T3         | 200                                      |
| T4         | 250                                      |
| T5         | 300                                      |
| T6         | 350                                      |
| T7         | 400                                      |
| T8         | 450                                      |
| T9         | 500                                      |

Figure 1. Effect of different foliar doses of micronutrients on shoot population density of Darjeeling tea (Average weekly mean data of five year during 2009 to 2014. Vertical bar indicate standard error of means.)
number of shoots m$^{-2}$. There were no significant differences in the number of banjhi/Dormant shoots m$^{-2}$ as a result of foliar application of Zn, B, Mn, and Mg in this study.

The changes in mean photosynthetic rate, stomata conductance, water use efficiency and transpiration under different foliar application of micronutrient are presented in table 3, respectively. When the data from all micronutrient treatments were combined, highest 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 (9.59 μ mol m$^{-2}$s$^{-1}$) but the lowest with the application of 400ml/ha Trasco-5 (Tea Special)( 9.03 μ 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 + 1% Mn + 1% B and minimum in Trasc-5 (Tea special) 0.21 mol m$^{-2}$s$^{-1}$. Maximum WUE was recorded in T6 (3.43 μ mol mmol$^{-1}$) and minimum in control T1 (2.09 μ mol mmol$^{-1}$). A positive correlation existed between WUE and $P_N$ (figure 3) which is in conformity with the findings of [12]. Micromix-5 treatment showed lowest rate of transpiration (E) then other treatments.

Table 3. Effect of foliar application of various micronutrients (Pure salt) and commercial product on photosynthetic rate ($P_N$), Stomatal conductance ($g_s$), Transpiration (E) and Water use efficiency (WUE), mean of five years.

| Treatments | Does | Physiological Parameters |
|------------|------|-------------------------|
|            |      | $P_N$ ($\mu$ mol m$^{-2}$s$^{-1}$) | $g_s$ (mol m$^{-2}$s$^{-1}$) | $E$ (m mol m$^{-2}$s$^{-1}$) | WUE ($\mu$ mol/ mmol$^{-1}$) |
| T1         | Control(No spray) | 8.89 | 0.27 | 3.87 | 2.29 |
| T2         | 2% Zinc | 10.58 | 0.25 | 3.84 | 2.76 |
| T3         | 1% Boron | 9.37 | 0.30 | 3.9 | 2.40 |
| T4         | 2% Zinc + 2% Magnesium | 10.49 | 0.29 | 4.01 | 2.61 |
| T5         | 2% Zinc + 1% Boron + 0.5% Molybdenum | 11.00 | 0.25 | 3.91 | 2.89 |
| T6         | 2% Zn + 2% Mg + 1% B | 12.27 | 0.26 | 3.59 | 3.41 |
| T7         | 2% Zn + 1% Mn + 1% B | 11.33 | 0.32 | 3.64 | 3.10 |
| T8         | Micromix-5 (1.5 kg/ha) | 9.18 | 0.25 | 3.43 | 2.71 |
| T9         | Trasco-5 (Tea special) - 400ml/ha | 9.03 | 0.21 | 3.46 | 2.60 |
|            | Sem ± | 0.27 | 0.01 | 0.05 | 0.07 |
|            | CD at 5% | 0.82 | 0.03 | 0.14 | 0.21 |

The chlorophyll is the most important plant pigment playing a vital role in determining the photosynthetic efficiency and productivity of the plant. The Chl content in leaves vary with the day length, irradiance and radiation quality, temperature and nutrient status of the soil [16]. The changes in total chlorophyll content under different doses of micronutrients presented in figure 2, respectively. From the results it was revealed that the content of chlorophyll was enhanced over the control. 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. While Trasco-5 (Tea special) and Micromix-5 both commercial product were less effective for the enhancing the chlorophyll contents in mature tea as compared to other micronutrients.
IV. CONCLUSION

There is a need to include the foliar application of micronutrients fertilizers in the manuring schedule of tea. It is suggested to apply Zinc sulphate, magnesium sulphate and boric acid through foliar at the rate of 2% + 2% + 1% (10kg/h + 10 kg/ha +5 kg/ha) to increase the productivity of tea. It is also observed that foliar application of micronutrients increased the primary metabolites like photosynthetic pigments. Finally a combination of zinc sulphate + magnesium sulphate + boric acid fertilizers can increase tea plantation yield. Hence, 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.
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