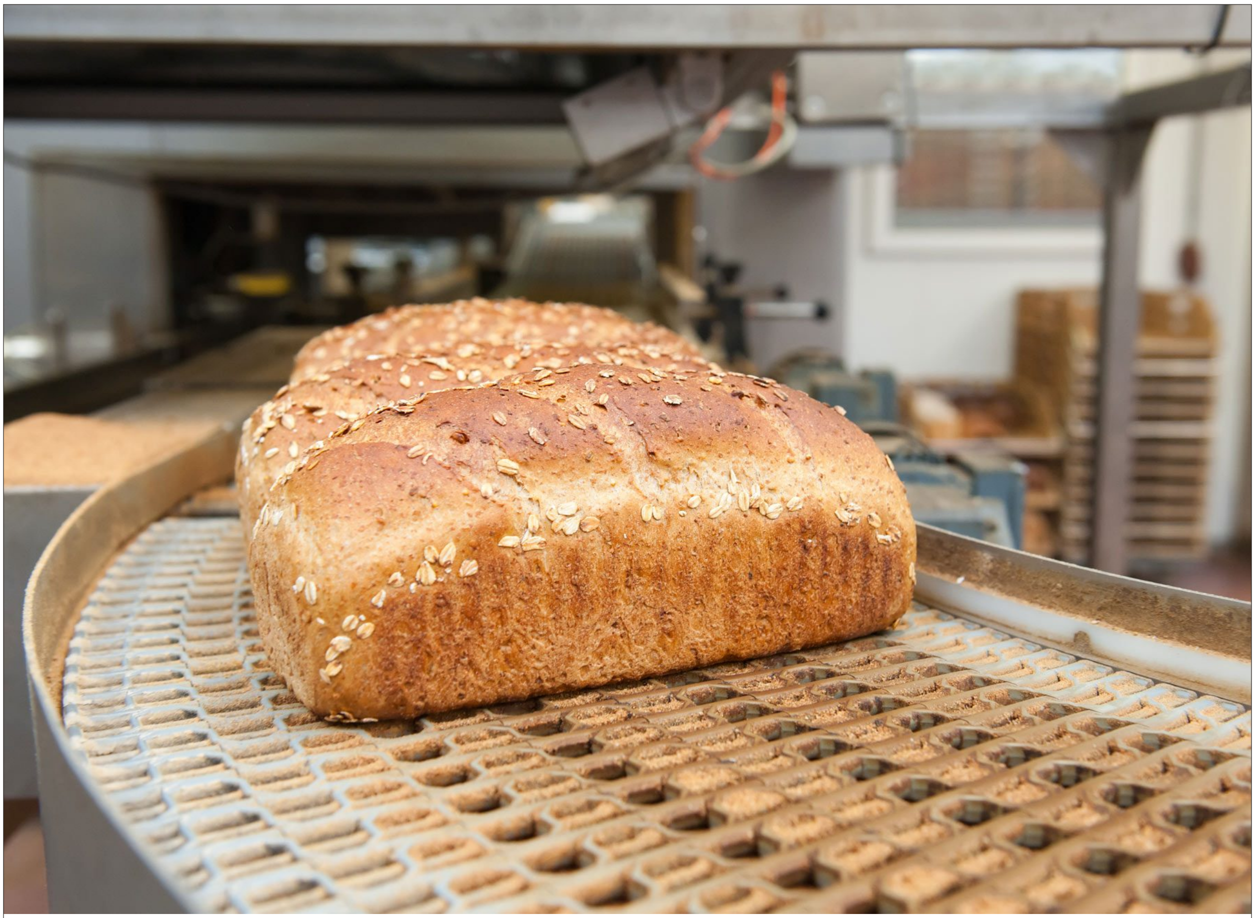

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# Impact of Magnesium Sulphate on Biochemical and Quality Constituents of Black Tea

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**Abstract:** Field experiment was laid out in Randomized Block Design (RBD) at UPASI tea experimental farm, with eight treatments and three replications. Amino acid content in tea leaves increased due to soil application of magnesium, but the 50% potassium reduction fields are reduced the amino acid content. The amino acid content significantly increased the soil applied magnesium when compared to the standard practices. The blocks which received the foliar application of micronutrient along with magnesium decreased the amino acid content when compared to the standard practices and control. The externally added magnesium increased the formation of 2-oxoglutarate and simultaneously another product aspartate/alanine was increased. The catechins content increased in 300 kg soil applied magnesium treatment and then decreased in 50% reduction of potassium fertilizer treatments. The TF value of made tea increased in 300 kg magnesium application plots and but decreased in the case of 50% reduction of potassium fertilizer. The control blocks having lower TF value when compared to the standard practices, because the K fertilizer is the important to increase the TF value. The maximum quantity of TF value obtained in the case of 300 kg soil applied blocks. The similar kind of trend was observed in TR values. Flavour index was made tea was higher in magnesium treated blocks, when compared to the control and NPK application. The magnesium content of tea leaves positively correlated with amino acid and amino acid transferase enzymes. This study further confirmed that antagonism exiting between K and Mg and synergism existing between P and Mg.

**Key words:** Biochemical parameters, quality parameters, magnesium, tea, potassium

## INTRODUCTION

Magnesium ranks third after N and K, in terms of the importance for economic growth of tea plants. It is the only mineral constituent in the chlorophyll molecule that regulates photosynthesis. In addition, it acts as an activator of many enzyme systems involved in carbohydrate metabolism and synthesis of nucleic acids and helps in translocation of sugar. On average, the harvestable tea crop contains between 0.05 and 0.25% Mg on dry weight basis (Othieno, 1992). Magnesium deficiency is characterized by the yellowing of old leaves and an inverted V shaped yellow tint, between the veins. There is premature leaf fall from the affected bushes. It is observed by plants as $Mg^{2+}$ ion. There is synergism between Mg and P and there is antagonism between Mg and K. The Mg content in most of the tea soils is generally low to medium and not adequate to maintain the high productivity of tea. To meet the magnesium nutrition in mature tea in Sri Lanka 500 kg of dolomitic lime is recommended after pruning once in a pruning cycle (Wickremasinghe and Krishnapillai, 1986). In

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North-East India besides application of dolomitic lime, foliar spray of magnesium sulphate at 1 to 2% concentration between autumn and spring (October to March) helps preventing Mg deficiency (Chakravartee and Sinha, 1995). Field trails on mature tea in Indonesia showed that the desirable rate of Mg was 25-50 kg $ha^{-1}$ as MgO with K:Mg ratio 3 to 4:1 and P:Mg ratio 0.5 to 1:1. The general fertilizer recommendations for tea soils of Indonesia having yield less than 4000 kg $ha^{-1}$ made tea is N:$P_2O_5$:$K_2O$:MgO: 5:0.5-1:2-3:0.5-1, for the maximum N rate of 360 kg $ha^{-1}$ (Wibowo, 1994). Higher level of potassium application along with nitrogen fertilizer reinforce the antagonism leading to magnesium deficiency was appeared in many parts of the south Indian tea gardens. It is an absolute necessity to come across the magnesium deficiency in high yielding fields to apply soil application of magnesium sulphate was recently recommended (Venkatesan, 2006).

There are reports stating that applications of magnesium fertilizers enhance the level of free amino acids in tea, but there is no substantial documents are appropriate to South Indian conditions. At this juncture, investigation on relation between Mg and total amino acids is warranted besides the amino acid synthesis pathway. Since, amino acids are responsible for flavour profile of made tea, it was noticed in Chinese tea gardens (Ma *et al*., 2005; Ruan *et al*., 1998, 1999). But there is no study in this grey area in particular reference to South Indian conditions. The widespread occurrence of flavonoids, proteins and lipids, as well as hydrolytic and oxidative enzymes, in tea leaf shoots plays an important role in the generation of much quality attributes during the manufacture of black tea. Tea aroma, which is composed of the Volatile Flavour Compounds (VFC) generated during tea processing, was recently demonstrated to be an important quality parameter determining the price of made tea. There is plenty of literature are available on influence of magnesium on flavour profile of made tea. But in the case of south Indian condition there is no study on this aspects and attempt was made it. In this article, the influence of magnesium sulphate application on biochemical composition of flush shoots and quality parameters studied with special reference to south Indian black teas.

## MATERIALS AND METHODS

Field experiment were laid out in Randomized Block Design (RBD) at UPASI tea experimental farm, with eight treatments and three replications; (1) Control (No fertilizer application), (2) NPK application as per standard recommendation to soil, (3) 200 kg Mg applied along with standard recommendation, (4) 300 kg Mg applied along with standard recommendation, (5) 200 kg Mg applied as per standard recommendation with 50% reduction of $K_2O$, (6) 200 kg Mg applied as per standard recommendation with 50% reduction of $K_2O$, (7) Foliar application of Mg at the 1% level along with micro nutrients and (8) Foliar application of Mg at the 2% level along with micro nutrients. The source of nitrogen was urea, source of potassium was muriate of potash (MOP), source of phosphorus is rock phosphate along with citric acid and source of magnesium was magnesium sulphate. The spray volume was kept at 200 L $ha^{-1}$ for last two treatments. Other nutrients and cultural practices were done at a uniform rate to all the treatments according to the standard recommendations of UPASI TRF (Verma and Palani, 1997). Leaf sampling was done during the first harvesting round of the year after fertilizer application. The experiment was conducted between 2005 and 2008 at UPASI Tea Experimental Farm, Valparai.

The biochemical constituents were carried out by collecting flush shoots, consisting of three leaves and a bud. A portion of collected leaf samples were subjected to analysis for total polyphenols (Dev Choudhury and Goswami, 1983), catechins (Swin and Hillis, 1958), total chlorophyll, carotenoids (Welburn, 1994) and free amino acids (Moore and Stein, 1948).

**Amino Transferase Enzymes**

About 1 g of plant tissue was homogenized with 0.2 M potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at 25,000 g for 15 min to get the enzyme fraction. To the treatment about 0.5 mL of aspartic acid substrate solution was added and warmed up in a water bath at 37°C for 3 min. Then 0.2 mL of enzyme extract was added and incubated at 37°C for 60 min and then 0.5 mL of dinitrophenylhydrazine solution was added. To the control about 0.5 mL of substrate, 0.5 mL of DNPH and 0.1 mL enzyme extract were added. The mixture was kept at room temperature for 20 min and then 0.4 N NaOH was added. After 10 min the absorbance was read at 510 nm (Bergmeyer, 1974).

The remaining sample was dried at 55°C and analyzed for potassium, magnesium and phosphorus content (Bhargava and Raghupathi, 2001).

Approximately, 2.5 kg of leaf samples were mini manufactured in the CTC unit and analyzed for theafalvins, thearubigins, total liquor colour and highly polymerized substance (Thanaraj and Seshadari, 1990). Volatile Flavour Compounds (VFC) were analyzed using a Gas Chromatograph (GC) (Perkin-Elmer Auto System XL). About 2.0 g of made tea sample were analyzed through a headspace sampler (Turbomatrix 16) of the GC for VFC using a Flame Ionization Detector (FID). A 60 m×0.25 cm i.d. capillary column was used. The column material was polyethylene glycol (HP-Innowax). The compounds were identified by comparison of their GC retention times with those of authentic chemicals (sigma). The flavour indeed (Obanda and Owuor, 1995) was calculated from the flavour profile, which is the ratio of the sum of VFC Group II to that of VFC group I.

The plot wise yield data were recorded and converted into made tea per hectare, using the following formula:

$$\text{Made tea yield} = \frac{(\text{Green leaf weight} \times \text{No. of bushes per hectare} \times 22.5)}{(\text{No. of bushes per plot} \times 100)}$$

Statistical analysis was carried out by the standard method (Gomez and Gomez, 1984). The correlation coefficients were worked out using Special Purpose Software for Statistics (Ver. 7.5).

## RESULTS AND DISCUSSION

**Biochemical Analysis**

The biochemical constituents were analyzed in two immediate harvesting rounds after manuring 2007. The statistically analyzed mean data are furnished in Table 1, along with statistical elements. Amino acid content in tea leaves increased due to soil application of magnesium, but the 50% potassium reduction fields are reduced the amino acid content. The amino acid content significantly increased the soil applied magnesium when compared to the standard practices. Statistical significance was noticed at the $p = 0.01$ level for the at the 300 kg application of magnesium fertilizer when compared to the NPK application and control treatments. The potassium content in soil stimulates the amino acid synthesis this kind of trend observed by Ruan *et al.* (1998). The blocks which received the foliar application of micronutrient along with magnesium decreased the amino acid content when compared to the standard practices and control; this is confirmed that potassium is required to nitrate reduction, which is an important step in nitrogen metabolism. About 300 kg magnesium sulphate applied in soil along with NPK fertilizer increased the polyphenol activity when compared to the control and standard practices. The foliar applied plots are also showed the

Table 1: Biochemical constituents of green leaf influenced by magnesium sulphate application

| Treatment details | Amino acids (%) | Poly-phenols (%) | Catechins | Chlorophyll ($g\ mg^{-1}$) | Carotenoids ($g\ mg^{-1}$) | Amino transferase enzymes Alanine@ | Aspartate@ |
|---|---|---|---|---|---|---|---|
| Control (no fertilizer application) | 1.10 | 20.2 | 18.4 | 1091 | 199 | 220 | 245 |
| NPK application as per standard recommendation (soil) | 1.67 | 22.1 | 18.9 | 1400 | 225 | 231 | 265 |
| 200 kg of Mg as per standard recommendation | 1.82 | 24.1 | 19.5 | 1620 | 191 | 245 | 275 |
| 300 kg of Mg as per standard recommendation | 1.92 | 25.1 | 20.1 | 1680 | 198 | 250 | 272 |
| 200 kg of Mg as per standard recommendation with 50% reduction of $K_2O$ | 1.58 | 22.4 | 19.6 | 1550 | 225 | 234 | 280 |
| 300 kg of Mg as per standard recommendation with 50% reduction of $K_2O$ | 1.66 | 22.9 | 19.7 | 1525 | 286 | 235 | 265 |
| Foliar application of Mg at 1% level along with micro nutrient | 0.96 | 24.1 | 24.0 | 1150 | 279 | 242 | 265 |
| Foliar application of Mg at 2% level along with micro nutrient | 1.18 | 24.3 | 22.7 | 1250 | 186 | 249 | 265 |
| Statistical analysis | | | | | | | |
| ±SEM | 0.19 | 0.67 | 1.11 | 60 | 10 | 7 | 9 |
| CD at p = 0.05 | 0.44 | 1.58 | 2.63 | 142 | 24 | 17 | 21 |
| CD at p = 0.01 | 0.65 | 2.34 | 3.89 | 210 | 35 | 25 | 32 |

similar kind of trend, because the micro nutrient application increased the polyphenols content. Magnesium is central metal atom of chlorophyll molecule; it directly influenced the chlorophyll content of tea leaves. The soil applied magnesium highly influenced the chlorophyll content and statistically significant at p = 0.01 level when compared to the standard practices. Similar kind of trend was observed in Ding *et al.* (2006). The catechin content increased in 300 kg soil applied magnesium treatment and then decreased in 50% reduction of potassium fertilizer treatments. The carotenoids content decreased due to the 50% reduction potassium fertilizer fields and foliar application fields.

## Enzyme Assay

The amino transferase enzyme catalyzes the following reaction in which the 2-oxoglutarate and aspartate/alanine are the final products.

$$\text{Glutamate+Oxaloacetate} \rightleftarrows \text{2-oxoglutarate+Aspartate/Alanine}$$

Both aspartate and alanine amino transferase enzymes increased soil applied and foliar application of magnesium treatments. The externally added magnesium increased the formation of 2-oxoglutarate and simultaneously another product aspartate/alanine was increased (Temple *et al.*, 1998; Kiss *et al.*, 2004). Because the influence of magnesium increased the protein synthesis and increased the accumulation of soluble organic nitrogen, nitrate nitrogen and amide forms of nitrogen in plant parts. Finally, the soluble amide forms of nitrogen converted into plant available form of amino acids.

## Quality Parameters

Influence of magnesium on quality of made tea are furnished in Table 2. Improvements of TF content of made tea could play an important role in price realization of South Indian black tea. The TF value of made tea increased in 300 kg magnesium application plots and but decreased in the case of 50% reduction of potassium fertilizer. The control blocks having

Table 2: Quality constituents of made tea and yield response influenced by magnesium sulphate application

| Treatment details | TF (%) | TR (%) | TF/TR | HPS (%) | TLC | FI | AA (%) | PP (%) | MTY (kg) |
|---|---|---|---|---|---|---|---|---|---|
| Control (no fertilizer application) | 1.07 | 11.99 | 1: 11.21 | 10.32 | 3.52 | 1.58 | 1.3 | 15.9 | 3820 |
| NPK application as per standard recommendation (soil) | 1.30 | 14.35 | 1: 11.04 | 10.42 | 4.01 | 1.75 | 1.42 | 17.43 | 4231* |
| 200 kg of Mg as per standard recommendation | 1.38 | 15.41 | 1: 11.17 | 10.77 | 4.15 | 2.42 | 1.51 | 17.33 | 4344** |
| 300 kg of Mg as per standard recommendation | 1.40 | 15.55 | 1: 11.11 | 10.78 | 4.25 | 2.52 | 1.53 | 17.23 | 4495** |
| 200 kg of Mg as per standard recommendation with 50% reduction of $K_2O$ | 1.35 | 14.75 | 1: 10.93 | 10.55 | 3.75 | 2.18 | 1.45 | 16.21 | 4245* |
| 300 kg of Mg as per standard recommendation with 50% reduction of $K_2O$ | 1.34 | 14.85 | 1: 11.08 | 10.56 | 3.68 | 2.08 | 1.46 | 16.45 | 4250* |
| Foliar application of Mg at 1% level along with micro nutrient | 1.33 | 15.21 | 1: 11.25 | 10.78 | 4.37 | 1.76 | 1.69 | 17.92 | 4355** |
| Foliar application of Mg at 2% level along with micro nutrient | 1.36 | 15.33 | 1: 11.10 | 10.78 | 4.45 | 1.56 | 1.72 | 18.21 | 4568** |
| Statistical analysis | | | | | | | | | |
| ±SEM | 0.04 | 0.26 | - | 0.12 | 0.06 | 0.40 | 0.05 | 0.19 | 155 |
| CD at p = 0.05 | 0.08 | 0.56 | - | 0.25 | 0.13 | 0.86 | 0.11 | 0.41 | 333 |
| CD at p = 0.01 | 0.11 | 0.78 | - | 0.35 | 0.18 | 1.19 | 0.15 | 0.57 | 462 |

*Significant at 5% level; **Significant at 1% level; CD: Critical difference; TF: Theaflavins; TR: Thearubigins; HPS: Highly polymerized substances; TLC: Total liquor colour; AA: Amino acids; PP: Polyphenols; FI: Flavour index; MTY: Made tea yield

lower TF value when compared to the standard practices, because the K fertilizer is the important to increase the TF value (Venkatesan *et al*., 2006). The maximum quantity of TF value obtained in the case of 300 kg soil applied blocks. The similar kind of trend was observed in TR values. The increase in TF value could be due to the increase in polyphenols at the optimum ratio, which is precursor of TF and TR. It increased in magnesium applied plots and decreased in 50% reduction blocks. The total amino acid content of made tea was significantly increased in soil applied magnesium plots and foliar applied plots. It confirmed that, magnesium induced the amino acid synthesis pathway (Ma *et al*., 2005). But in the case of 50% reduction fields slightly decreased the amino acid content of made tea. Highly polymerized substances are believed to be part of TR and responsible for the body, strength richness and colour of the liquor. HPS content slightly increased in soil applied magnesium and foliar applied blocks. Total liquor colour recorded in all the treatments including control was above 3.0, which is the minimum boundary for South Indian CTC teas. But in the case of foliar applied blocks are having maximum TLC value (>4.0), it is due to the combination of micro nutrient application. The flavour index of made tea directly reflected in price realization (Ravichandran and Parthiban, 1998; Rawat and Gulati, 2008). It was higher in magnesium treated blocks, when compared to the control and NPK application. Because flavour is dependent on the availability of precursors present in tea shoot, stimulation of conditions during tea manufacture for their liberation and retention of flavour components in the product. Amino acid is precursor of flavour profile; already in the whole experiment amino acid is very much pronounced the external application of magnesium fertilizer.

**Nutrient Content of Tea Leaves**

The nutrient content of tea leaves were analyzed and furnished in Table 3. The magnesium content of shoots and mature leaf significantly at p = 0.01 level increased in all treatment when compared to the control and standard practices. The foliar applied blocks having higher magnesium content in mature leaf. The reduction of potassium fertilizer fields are yielded high amount of magnesium, this could be because of antagonism between

Table 3: Green leaf nutrient content influenced by magnesium sulphate application

| Treatment details | Magnesium | | Potassium | | Phosphorus | |
|---|---|---|---|---|---|---|
| | ML | 3L + B | ML | 3L + B | ML | 3L + B |
| Control (no fertilizer application) | 0.167 | 0.197 | 1.25 | 1.70 | 0.105 | 0.195 |
| NPK application as per standard recommendation (soil) | 0.175 | 0.210 | 1.54** | 1.90** | 0.133 | 0.263* |
| 200 kg of Mg as per standard recommendation | 0.190* | 0.255* | 1.35* | 1.86* | 0.127* | 0.247* |
| 300 kg of Mg as per standard recommendation | 0.192* | 0.275** | 1.45* | 1.86* | 0.128* | 0.257* |
| 200 kg of Mg as per standard recommendation with 50% reduction of $K_2O$ | 0.195* | 0.285** | 1.31 | 1.72 | 0.130* | 0.248* |
| 300 kg of Mg as per standard recommendation with 50% reduction of $K_2O$ | 0.210** | 0.290** | 1.30 | 1.70 | 0.129* | 0.259* |
| Foliar application of Mg at 1% level along with micro nutrient | 0.223** | 0.274** | 1.28 | 1.62 | 0.121 | 0.238 |
| Foliar application of Mg at 2% level along with micro nutrient | 0.226** | 0.276** | 1.27 | 1.63 | 0.120 | 0.236 |
| Statistical analysis | | | | | | |
| Sem± | 0.01 | 0.02 | 0.04 | 0.05 | 0.01 | 0.02 |
| CD at p = 0.05 | 0.02 | 0.05 | 0.09 | 0.12 | 0.02 | 0.05 |
| CD at p = 0.01 | 0.03 | 0.07 | 0.14 | 0.17 | 0.03 | 0.07 |

*Significant at 5% level; **Significant at 1% level; CD: Critical difference; ML: Mature leaf

Table 4: Correlation coefficients among biochemical, enzyme activity and nutrient content of green leaves

| Content | Amino acids | Amino transferase | | Carotenoids | Catechins | Chlorophyll | K | Mg | P |
|---|---|---|---|---|---|---|---|---|---|
| | | Alanine | Aspartate | | | | | | |
| Alanine | 0.998** | | | | | | | | |
| Aspartate | 0.917 | 0.923* | | | | | | | |
| Carotenoids | -0.421 | -0.368 | -0.123 | | | | | | |
| Catachins | 0.973* | 0.981* | 0.851 | -0.349 | | | | | |
| Chlorophyll | 0.980* | 0.986* | 0.971* | -0.239 | 0.951 | | | | |
| K | 0.635 | 0.667 | 0.866 | 0.390 | 0.607 | 0.778 | | | |
| Mg | 0.986* | 0.983* | 0.840 | -0.493 | 0.985* | 0.990** | -0.526 | | |
| P | 0.677 | 0.713 | 0.867 | 0.371 | 0.677 | 0.810 | 0.990 | 0.586 | |
| Polyphenols | 0.994** | 0.999** | 0.923 | -0.330 | 0.986* | 0.988* | 0.686 | 0.980* | 0.735 |

*Significant at 5% level; **Significant at 1% level; K: Potassium; Mg: Magnesium; P: Phosphorus

potassium and magnesium (Kirkby and Mengal, 1976; Verma and Palani, 1997; Venkatesan *et al*., 2006; Jayaganesh and Venkatesan, 2006). The potassium content of tea leaves was decreased in foliar applied plots and 50% reduction fields.

The phosphorus content of tea leaves very much pronounced with magnesium nutrition. It is positively correlation with magnesium, because the soluble form of magnesium phosphate complex should be form. P content increased significantly at p = 0.05 level when compared to the control treatments. This study confirmed that both the nutrition's are having synergism effect. The made tea yield recorded between November 2005 and October 2007, is given in Table 4. Magnesium application positively influenced the made tea yield. However, the statistical significant was noted at p = 0.01 level in foliar application plots and soil application of magnesium along with standard recommendation. The 50% reduction of potassium fertilizer blocks decreased the yield, when compared to the above treatments. Achievement of higher yield in magnesium applied plots, due to magnesium sulphate application, could be because sulphur must have entered into a crucial role in the nitrogen metabolism, which is in line with findings created by Ruan *et al*. (1998).

## Correlation Coefficients

The magnesium content of tea leaves positively correlated with amino acid and amino acid transferase enzymes. It is clearly noted that magnesium nutrition involved the amino acid synthesis metabolism. The similar kind of trend observed in chlorophyll molecule also and correlated at p = 0.01 level. Because the magnesium is the only metal present in the

Table 5: Correlation coefficients among quality parameters and nutrient content of green leaves

| | AA | FI | HPS | K | Mg | P | PP | TF | TLC |
|---|---|---|---|---|---|---|---|---|---|
| FI | 0.943* | | | | | | | | |
| HPS | 0.949* | 0.997** | | | | | | | |
| K | 0.774 | 0.520 | 0.543 | | | | | | |
| Mg | 0.941* | 0.991** | 0.980* | -0.526 | | | | | |
| P | 0.805 | 0.564 | 0.577 | 0.990** | 0.586s | | | | |
| PP | 0.846 | 0.624 | 0.649 | 0.989** | 0.618 | 0.978* | | | |
| TF | 0.983* | 0.867 | 0.878 | 0.877 | 0.866 | 0.897 | 0.928 | | |
| TLC | 0.982* | 0.866 | 0.873 | 0.872 | 0.875 | 0.901 | 0.919 | 0.997** | |
| TR | 0.990** | 0.887 | 0.899 | 0.855 | 0.883 | 0.874 | 0.913 | 0.999** | 0.995** |

*Significant at 5% level; **Significant at 1% level; CD: Critical difference; TF: Theaflavins; TR: Thearubigins; HPS: Highly polymerized substances; TLC: Total liquor colour; AA: Amino acids; PP: Polyphenols; FI: Flavour index; MTY: Made tea yield

chlorophyll molecule. The magnesium content of tea leaves negatively correlated with carotenoids content. The Mg content in leaf was negatively and significantly correlated with potassium. This study further confirmed that antagonism exiting between K and Mg. Potassium and phosphorus having significantly positive correlation coefficients, it confirmed that synergism existing between P and K. Total amino acid content in harvestable shoots positively significant at $p = 0.01$ level in aspartate amino transferase enzymes.

Similarly, the correlation coefficients worked out among made tea yield, green leaf nutrients and quality parameters are provided in Table 5. Magnesium content was positively correlated with flavour index of made tea at $p = 0.05$ level. It indicates that soil applied magnesium increased the flavour of made tea and the result confirmed the increase in price of tea. Similar kind of trend observed in HPS also. Total amino acid content of made tea positively significant with TF, TR, HPS and flavour index, because amino acid content was precursor of volatile compounds (Ravichandran and Parthiban, 1998).

## CONCLUSION

This study confirmed that soil application of magnesium sulphate along with NK application is viable one. In this practices the yield, quality constituents of made tea and tea shoots should be increased and simultaneously price of the made tea also increased. This study recommended that 300 kg of magnesium sulphate applied in soil as two split in year $ha^{-1}$ applied in April/May and November/December in high yielding fields.

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