



Effect of Plant Density and Nitrogen Fertilizer Application Rates on Nutrient Content of Clonal Tea Leaf

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JW and DK designed the study and wrote the protocol. Author KS wrote the first draft of the manuscript, managed the literature searches and analyses of the study. Author KK sampled and prepared the leaf for analysis. Author KL performed the spectroscopy analysis. All authors read and approved the final manuscript.

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ABSTRACT

Trial on plant density which started in 1990 has revealed that in clonal tea, yield significantly increased with decrease in plant population density (ppd), with the highest ppd showing significantly lower yield than all the other ppd. However, this effect was opposite when the tea was young. Nitrogen availability affects yield of tea. Yields increase with increasing use of nitrogen up to high levels with proportional increase in economic returns. It was not therefore known whether the same ppd and nitrogen effect applies to nutrients content. This study was carried out to determine the effect of plant density of AHP S15/10 clonal tea plants and rates of nitrogenous fertilizer applied on leaf nutrients content in Kericho, Kenya. Leaf samples were collected from all the experimental plots. The samples were analyzed for their contents of macro and micro elements by ICPE spectroscopy. The tea leaves of the study clone showed similar accumulation patterns for nitrogen, potassium, calcium, magnesium, manganese and aluminium nutrients across the nitrogen rates.

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Nitrogen content was least in the highest ppd. Among the nutrients content in the leaf tissue, phosphorus was the most abundant element (0.21 – 0.24%). This therefore means that plant density determine the nutrients content in leaf.

Keywords: Tea; nitrogen; nutrient; plant population density.

1. INTRODUCTION

Tea is one of the most popular beverages and an important commercial crop spread over many areas of the world [1]. Yield of leaf plucked from a field of tea is dependent among many factors, up on the extent to which the field is covered by the bushes. Cultural practices such as planting density have effects on yield [2]. Tea is normally planted in single or double rows separated by 1.2 to 1.8 m between the rows to allow access to the bushes for plucking and maintenance [3]. Dense planting can improve the early yield and revenue, encouraging early establishment of plantlets and appropriate ground cover, reducing the cost of weeding and minimizing water losses by decrease of surface evaporation and run off [4,5]. The yield gradually decreases with higher density as the plants continue to age. Trial on plant density experiment started in 1990 at TRI Kericho, Kenya has revealed that in clonal tea, yield significantly increased with decrease in plant population density (ppd), with the highest ppd showing significantly lower yield than all the other ppd after a certain time. Nitrogen availability affects yield of tea. Yields increase with increasing use of nitrogen up to high levels with proportional increase in economic returns [6]. Generally, tea plants need 40 to 200 kgNha⁻¹yr⁻¹ during the years of growth [3]. The production of new shoots and content of nitrogenous compounds, such as free amino acids, which are major quality indicator of green tea, is increased by application of nitrogen fertilizer [7]. Quality indicators of green tea such as amino acid and protein content are influenced by soil fertility [8]. The main sources of micronutrients in plants are soil and agro inputs [9,10]. Plants take up the elements from the soil and under certain conditions, high levels can be accumulated in the leaves [11,10]. Several elements such as Ca, Na, K, Mg and Mn are present in mg/g quantities while elements such as Cr, Fe, Co, Ni, Cu, Zn, Se and Cd are present in µg/g level [12,13,10]. It is not therefore known whether the same effect applies to nutrients content. Therefore, it was deemed that the determination of effect of plant density of AHP S15/10 clonal tea plants and rates of nitrogenous fertilizer applied on concentration of macro and

micronutrients in tea leaves could help enhance the sustainability of tea cultivation.

2. METHODS AND MATERIALS

2.1 Site Description and Experimental Layout

The research used an existing experiment at KALRO, Tea Research Institute, Timbilil estate, Kericho Kenya. The experiment was started in 1990 using clone AHP S15/10 tea planted in 1988 on a cleared virgin forest area. The site is situated at an altitude of 2178 m asl, latitude 0° 22' S and longitude 35° 21' E and was a virgin forest prior to the start of experiment. The experimental design was a split-plot completely randomized block design with four main treatments (as plant population density i.e. 26896 (2x2); 13448 (2x4); 8975 (3x4) and 6730 (4x4) plants ha⁻¹) and four sub-treatments (as rates of nitrogen i.e. 80, 160, 240 and 320 kg ha⁻¹ year⁻¹ as NPKS 25:5:5:5) replicated three times.

2.2 Leaf Samples

Mature tea leaves were sampled from every plot of the 48 plots involved (about 100 g), taken to the lab, dried in an oven at temperature of 105°C before milling.

2.3 Chemical Analysis

Total nitrogen concentration was determined by the micro-Kjeldahl method [7]. 0.1 g of milled samples were digested for an hour in concentrated H₂SO₄ plus 1/4 catalyst tablet. After cooling, the digest was made alkaline with 40% NaOH solution and the NH₃ distilled was collected in 10 ml boric acid containing mixed indicator. Total N was determined by titrating the distillate against 0.035 M HCl.

For the other elements i.e. phosphorus, potassium, calcium, magnesium and trace elements (Fe, Cu, Zn, Mn), exactly 0.25 g of the dried and ground tea leaf sample was ashed for four hours and then digested using 2 parts of 1:1 mixture of concentrated HNO₃ (69–70.5%) and concentrated HCl (37%) under reflux to 3 parts of

hydrogen peroxide [14]. 5 mls is then pipetted to 25 mls volumetric flask. 5 mls strontium chloride is added and topped to 25 mls mark. Concentrations of the elements is then determined using ICPE.

Nutrient element contents of tea plants were evaluated according to critical values [15,16].

2.4 Statistical Analysis

The data were subjected to the analysis of variance (ANOVA) using the MSTATC software package [17]. In case of significant treatment effects, a comparison of means was performed by means of least significant difference (LSD) method at a significance level of 5% ($p = 0.05$). Use of difference between treatments implies statistical difference ($P = 0.05$) while no difference implies no statistical difference.

3. RESULTS AND DISCUSSION

3.1 Leaf Samples Results

Effect of plant population density and nitrogen fertilizer rates on mature leaf nitrogen, phosphorus, potassium, calcium, magnesium, manganese, aluminium, copper, iron and zinc are presented in Table 1. The results of the studied nutrient in the mature leaves of clones AHP S15/10) show the ability of the clone to accumulate high amounts of both macro- and micronutrient elements.

The results show reduced nutrients level with increased population densities. This indicates that different population densities have different abilities to absorb various nutrients from the soils. There was significant difference in N with increase in fertilizer rates ($LSD_{0.05}=0.17$). This was expected due to increase N level in the soil through increased supply with application rates. Spacing 2X4 gave the highest uptake at the rate of $320 \text{ kgNha}^{-1}\text{yr}^{-1}$ followed by 3X4 being high at the rate of $160 \text{ kgNha}^{-1}\text{yr}^{-1}$. The spacing 2X2 records the lowest uptake at the rate of $80 \text{ kgNha}^{-1}\text{yr}^{-1}$. This is because of the high population density hence competition for uptake of nitrogen. [18], reported that the response of nitrogen at any level depends on the availability of other nutrients. Nitrogen remains one of the main nutrients required for plant growth and yield [19]. Phosphorus showed significant difference with population density ($LSD_{0.05}=0.01$). Spacing

2X2 gives the highest percentage of phosphorus at the rate of $240 \text{ kgNha}^{-1}\text{yr}^{-1}$ while spacing 3X4 gives the lowest percentage of phosphorus at the rate of $160 \text{ kgNha}^{-1}\text{yr}^{-1}$. Potassium had significant difference due to both population density and fertilizer rates ($LSD_{0.05}=0.06$ for both) with spacing 2X2 recording the highest potassium uptake. There was a significant interaction between the population density and different fertilizer rates ($LSD_{0.05}=0.049$) meaning that the responses did not occur in the same pattern. Calcium uptake was significantly different due to population density ($LSD_{0.05}=0.023$) and nitrogen fertilizer rates ($LSD_{0.05}=0.024$). Spacing 2X2 showed the highest uptake of calcium into the leaf at the rate of $80 \text{ kgNha}^{-1}\text{yr}^{-1}$. The tea plants in the spacing 3X4 absorbed the lowest calcium at the rate of $160 \text{ KgNha}^{-1}\text{yr}^{-1}$. Significant difference due to fertilizer rates ($LSD_{0.05}=0.01$) was also observed in magnesium with spacing 4X4 recording approximately the same amount of magnesium at every rate. The spacing 2X2 recorded the highest uptake in almost every rate of fertilizer application. The mean level of manganese varied significantly with fertilizer rates ($LSD_{0.05}=0.02$). Spacing of 4X4 recorded the highest manganese uptake at every rates of application. This is because of the low population density meaning that every tea plant got adequate amount of manganese. Aluminium had significant difference due to fertilizer rates ($LSD_{0.05}=84.52$) with spacing 2X2 showing the highest amount of aluminium at every rates of fertilizer application. There was no significant difference in copper, Iron and Zinc.

The results presented herein demonstrate that different population densities have varied abilities to absorb nutrients in single locations and same clone has different ability to absorb the nutrients from different fertilizer rates. Therefore, the most effective planting density should be determined from the interaction of various technical and financial factors [4]. The concentration of nutrients element of tea leaf are related with the soil environment. Fresh tea leaf has a mineral content which may vary depending on the soil on which it is grown, maintenance and fertilizing. [6], reported that plant nutrient concentrations in the tea plant are highest in the young leaves and buds with concentration ranges for the major nutrient elements K (20.9–23.6 mg/g), Ca (4.4–4.7 mg/g) and Mg (2.0– 2.3 mg/g). These ranges of values are slightly higher than the levels obtained in the mature leaves of the studied tea clone.

Table 1. Long term effect of plant population and N-fertilizer rates on mature leaves nutrients content

| Period | Plant population per ha (or spacing in sq. m) | Fertilizer rates (kg N/ha/year) | | | | Plant population mean |
|--------|--|---------------------------------|-------|-------|-------------------|-----------------------|
| | | 80 | 160 | 240 | 320 | |
| %N | 26896 (2x2) | 2.32 | 2.55 | 2.72 | 2.47 | 2.52 |
| | 13448 (2x4) | 2.62 | 2.77 | 2.73 | 3.09 | 2.8 |
| | 8975 (3x4) | 2.74 | 3.01 | 2.8 | 3.02 | 2.89 |
| | 6730 (4x4) | 2.72 | 2.93 | 2.94 | 2.95 | 2.88 |
| | N-rate mean | 2.59 | 2.82 | 2.8 | 2.88 | (2.77) |
| | | <u>N-rates</u> | | | <u>PP*N-rates</u> | <u>PP</u> |
| | | C.V.(%) | 10.29 | | 10.29 | 8.68 |
| | LSD (P<0.05) | 0.17 | | NS | NS | |
| %P | 26896 (2x2) | 0.217 | 0.22 | 0.24 | 0.223 | 0.225 |
| | 13448 (2x4) | 0.223 | 0.217 | 0.237 | 0.23 | 0.227 |
| | 8975 (3x4) | 0.227 | 0.21 | 0.22 | 0.223 | 0.22 |
| | 6730 (4x4) | 0.227 | 0.213 | 0.227 | 0.23 | 0.224 |
| | N-rate mean | 0.223 | 0.215 | 0.231 | 0.227 | 0.224 |
| | | <u>N-rates</u> | | | <u>PP*N-rates</u> | <u>PP</u> |
| | | C.V.(%) | 5.67 | | 5.67 | 0 |
| | LSD (P<0.05) | NS | | NS | 0.01 | |
| %K | 26896 (2x2) | 1.43 | 1.35 | 1.49 | 1.37 | 1.41 |
| | 13448 (2x4) | 1.26 | 1.15 | 1.35 | 1.24 | 1.25 |
| | 8975 (3x4) | 1.33 | 1.14 | 1.16 | 1.28 | 1.22 |
| | 6730 (4x4) | 1.28 | 1.08 | 1.25 | 1.21 | 1.21 |
| | N-rate mean | 1.32 | 1.18 | 1.31 | 1.27 | 1.27 |
| | | <u>N-rates</u> | | | <u>PP*N-rates</u> | <u>PP</u> |
| | | C.V.(%) | 8.25 | | 8.25 | 7.04 |
| | LSD (P<0.05) | 0.06 | | NS | 0.06 | |
| %Ca | 26896 (2x2) | 0.62 | 0.46 | 0.6 | 0.56 | 0.56 |
| | 13448 (2x4) | 0.52 | 0.42 | 0.46 | 0.43 | 0.46 |
| | 8975 (3x4) | 0.42 | 0.39 | 0.4 | 0.4 | 0.4 |
| | 6730 (4x4) | 0.4 | 0.41 | 0.41 | 0.4 | 0.4 |
| | N-rate mean | 0.49 | 0.42 | 0.47 | 0.44 | 0.46 |
| | | | | | | |

| | | | | | | |
|----------------|---------------------|----------------|--------|-------------------|--------|-----------|
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 8.94 | | 8.94 | | 6.93 |
| | LSD (P \leq 0.05) | 0.024 | | 0.049 | | 0.023 |
| %Mg | 26896 (2x2) | 0.217 | 0.193 | 0.207 | 0.193 | 0.203 |
| | 13448 (2x4) | 0.183 | 0.17 | 0.18 | 0.177 | 0.177 |
| | 8975 (3x4) | 0.163 | 0.14 | 0.14 | 0.143 | 0.147 |
| | 6730 (4x4) | 0.16 | 0.137 | 0.143 | 0.137 | 0.144 |
| | N-rate mean | 0.181 | 0.16 | 0.167 | 0.162 | 0.168 |
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 9.56 | | 9.56 | | 0 |
| | LSD (P \leq 0.05) | 0.01 | | NS | | NS |
| %Mn | 26896 (2x2) | 0.177 | 0.152 | 0.177 | 0.168 | 0.169 |
| | 13448 (2x4) | 0.204 | 0.197 | 0.198 | 0.203 | 0.2 |
| | 8975 (3x4) | 0.234 | 0.232 | 0.269 | 0.257 | 0.248 |
| | 6730 (4x4) | 0.262 | 0.259 | 0.277 | 0.294 | 0.273 |
| | N-rate mean | 0.219 | 0.21 | 0.23 | 0.23 | 0.222 |
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 12.22 | | 12.22 | | 0.2 |
| | LSD (P \leq 0.05) | 0.02 | | NS | | NS |
| Al(ppm) | 26896 (2x2) | 1413.33 | 1006 | 1206.67 | 1063 | 1172.25 |
| | 13448 (2x4) | 1042.33 | 962.67 | 1040.33 | 961.67 | 1001.75 |
| | 8975 (3x4) | 902 | 794 | 886 | 821 | 850.75 |
| | 6730 (4x4) | 958 | 935.67 | 909 | 981.67 | 946.17 |
| | N-rate mean | 1078.92 | 924.58 | 1010.58 | 956.83 | 992.73 |
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 14.29 | | 14.29 | | 20.40 |
| | LSD (P \leq 0.05) | 84.52 | | NS | | NS |
| Cu(ppm) | 26896 (2x2) | 28.33 | 30.33 | 45.33 | 37 | 35.25 |
| | 13448 (2x4) | 31 | 28 | 31.67 | 32 | 30.67 |
| | 8975 (3x4) | 30 | 36.67 | 26.67 | 29.67 | 30.75 |
| | 6730 (4x4) | 29.67 | 25.67 | 32 | 25.67 | 28.25 |

| | | | | | | |
|----------------|---------------------|----------------|--------|-------------------|--------|-----------|
| | N-rate mean | 29.75 | 30.17 | 33.92 | 31.08 | 31.23 |
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 25.86 | | 25.86 | | 32.76 |
| | LSD (P \leq 0.05) | NS | | NS | | NS |
| Fe(ppm) | 26896 (2x2) | 101.67 | 126.33 | 208 | 163.67 | 149.92 |
| | 13448 (2x4) | 179 | 132.33 | 182.33 | 133 | 156.67 |
| | 8975 (3x4) | 115.67 | 110 | 136.67 | 194.33 | 139.17 |
| | 6730 (4x4) | 109 | 167 | 122 | 151 | 137.25 |
| | N-rate mean | 126.33 | 133.92 | 162.25 | 160.5 | 145.75 |
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 29.53 | | 29.53 | | 47.64 |
| | LSD (P \leq 0.05) | NS | | NS | | NS |
| Zn(ppm) | 26896 (2x2) | 41 | 69 | 53.33 | 58 | 55.33 |
| | 13448 (2x4) | 49 | 61.67 | 49.67 | 47 | 51.83 |
| | 8975 (3x4) | 55 | 44.67 | 54.33 | 88.33 | 60.58 |
| | 6730 (4x4) | 57.67 | 62 | 45.67 | 54 | 54.83 |
| | N-rate mean | 50.67 | 59.33 | 50.75 | 61.83 | 55.65 |
| | | <u>N-rates</u> | | <u>PP*N-rates</u> | | <u>PP</u> |
| | C.V.(%) | 18.52 | | 18.52 | | 25.16 |
| | LSD (P \leq 0.05) | NS | | 6.14 | | NS |

The levels of nutrients in the studied mature leaf according to [20] was due to the fact that nutrient elements such as N, P, K, S and Mg are highly mobile in the tea plant tissue and are translocated from old leaves to young leaves. [21], suggested the ability of the tea plant to accumulate heavy metals particularly Mn, Fe and Zn, to a lesser extent Cu. Ranges of levels of Fe, Zn and Cu in the tea leaf were reported to be 137.25– 156.67 mg/kg, 51.83–60.58 mg/kg, and 28.25–35.25 mg/kg, respectively. [22], have reported higher Mn contents (1100–2678 mg/kg) in tea leaves from Turkey and Japan.

4. CONCLUSION

The results show reduced nutrients level with increased population densities. This indicates that different population densities have varied abilities to absorb nutrients in single locations and same clone has different ability to absorb the nutrients from different fertilizer rates. Therefore, the most effective planting density should be determined from the interaction of various technical and financial factors. Knowledge and understanding of the importance of the cultural practices such as planting density and mineral nutrition (nitrogen fertilization) on the nutrients content of tea is significant.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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