

STUDIES ON THE MINERAL NUTRITION OF TEA 4—AMMONIUM AND NITRATE AS FORMS OF NITROGEN FOR TEA PLANTS IN SAND CULTURE

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Ammonium sulphate is the most widely-used nitrogenous fertilizer for tea in Ceylon. It is probable that in the near future, increasing quantities of urea will be employed.

The present study was designed to evaluate the relative performance of tea plants in sand culture, supplied with their nitrogen exclusively in the nitrate or ammonium form or as varying combinations of the two forms. Between the extreme treatments which consisted of either ammonium or nitrate as the only source of nitrogen were nine mixtures of ammonium and nitrate, separated by steps of ten %. As the nitrate content rose the ammonium was reduced by an equivalent quantity so that the total nitrogen supplied remained constant for all treatments.

Comprehensive growth assessments were carried out on the plants after fourteen months of treatment. Nitrogenous constituents in leaf samples were categorized into ammonium nitrate, amino acid, amide and total nitrogen. Plants which had received their nitrogen as 60, 70 or 80% in the nitrate form were superior in growth while those that had received 0, 10 and 20% performed poorly. The other treatments were intermediate. Determinations of the nitrogenous fractions revealed differences between treatments.

The analytical data are discussed in relation to the growth and performance of the experimental plants.

Experiments with a number of plant species where ammonium and nitrate nutrition have been compared have shown that the form of nitrogen supply exerts a pronounced effect on both the growth and the chemical composition of the plant (eg Clark 1936; Sideris, Krauss & Young 1938; Arnon 1939; Grassmanis & Leeper 1967; Kirkby & Mengel 1967; Kirkby 1968). Previous work done in this field on tea has been described in the preceding paper by Kularatne and Bhavanandan. Our work represents an extension of their studies but differs in two main respects :

- a* — the experimental plants were maintained under differential treatments for a longer period of time, and
- b* — in addition to the extreme treatments embodying exclusively one form or the other of nitrogen, a graded series of combinations were also included as treatments.

The primary objective of this study was to evaluate the effects of the form of nitrogen nutrition on growth of tea plants under carefully-controlled environmental conditions. Analyses of leaf samples for the different nitrogen fractions were also carried out and are discussed.

MATERIALS AND METHODS

The experimental plants were grown from cuttings of the clone Drayton 1. They were initially in soil and uniform plants were transplanted into the pots with sand when they were one year old. For this operation, the roots were carefully washed free of all soil. For the first three weeks following transplanting, the plants received only demineralized water at a frequency and volume sufficient to ensure moist conditions during this initial stage of establishment. For the next ten weeks, all the plants were supplied with an uniform nutrient solution (Hewitt's solution of quarter-strength, pH 3.5). For details of the composition of this solution reference may be made to Pethiyagoda, Krishnapillai & Nagarajah 1969.

When the differential treatments were commenced at 13 weeks after transplanting, nutrient solutions equivalent to Hewitt's solution at half strength were employed. The experiment consisted of eleven treatments. Between the two extreme treatments where either ammonium or nitrate was the only source of supply of nitrogen, there were nine mixtures of the two forms, separated by steps of 10 ppm. As the nitrate nitrogen content rose by 10 ppm, the ammonium dropped by a corresponding quantity, thereby maintaining the total nitrogen level at 100 ppm. Each plant received a constant volume of nutrient solution at each application. The eleven treatments were replicated five times and the pots were arranged in the glass house in a randomized block design. All pots were leached out with demineralized water once each week, as a precaution against accumulation of salts in the sand. The ammonium ion was supplied in ammonium sulphate while the nitrate ion was provided by appropriate combinations of calcium nitrate and potassium nitrate according to treatment. The sulphates of calcium and potassium were used as balancing salts in quantities designed to maintain concentrations of the cations at a constant level. Tables 1 & 2 outline the manner in which the treatment solutions were composed and Table 3 presents their ionic composition.

TABLE 1 — *Composition of stock solutions and dilutions required before use—Basal nutrients common to all treatments*

Salt	Stock solution (g/l)	Required dilution (times)
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	208	2000
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	185	500
Ferric citrate ($5\text{H}_2\text{O}$)	16.8	1000
$\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$	11.7	2000

Micronutrient solution to all treatments

TABLE 2 — *Composition of stock solutions—Nitrogen supplying and cation balancing salts*

(a) *Composition*

Salt	Stock solution (g/L)
$(\text{NH}_4)_2\text{SO}_4$	118
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	147.6
KNO_3	63.2
K_2SO_4	54.5
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	Appropriate quantities added as the solid to diluted solutions

(b) *Dilutions (as ml stock solution/20 l; CaSO_4 as g/20 l)*

Treatment No.	$(\text{NH}_4)_2\text{SO}_4$	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	KNO_3	K_2SO_4	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
1	80	0	0	80	8.6
2	72	11.4	0	80	7.35
3	64	22.9	0	80	6.15
4	56	34.3	0	80	4.90
5	48	45.7	0	80	3.69
6	40	57.1	0	80	2.45
7	32	68.6	0	80	1.25
8	24	80.0	0	80	0
9	16	80.0	22.9	57.1	0
10	8	80.0	45.7	34.5	0
11	0	80.0	68.6	11.5	0

TABLE 3 — *Ionic composition of nutrient solution (all figures as ppm)*

Treatment	Total NH ₄ -N	NO ₃ -N	(NH ₄) ₂ SO ₄ NH ₄ -N	Ca(NO ₃) ₂ .4H ₂ O NO ₃ -N.Ca	KNO ₃ NO ₃ -N.K	K ₂ SO ₄ K	CaSO ₄ .2H ₂ O Ca
1	100	0	100	—	—	98	100
2	90	10	90	10	14	98	85
3	80	20	80	20	28	98	72
4	70	30	70	30	43	98	57
5	60	40	60	40	57	98	43
6	50	50	50	50	72	98	28
7	40	60	40	60	85	98	15
8	30	70	30	70	100	98	—
9	20	80	20	70	100	10	28
10	10	90	10	70	100	20	56
11	0	100	0	70	100	30	84
						14	—

The treatments were continued for a period of fourteen months by which time the plants in the best treatments had grown to a height of about 2 m. The single assessment was carried out at this stage.

RESULTS AND DISCUSSION

General observations

Visual differences between experimental plants were evident within two months of commencement of the differential treatments. Plants receiving their nitrogen mainly or exclusively as ammonium, produced darker green leaves than those that were supplied a greater proportion of their nitrogen as nitrate. Plants toward both ends of the treatment-range showed abnormalities. Within three months those receiving mainly ammonium nitrogen, displayed a characteristic chlorosis of their leaves, associated with prominent crinkling and recurving along the stem axis. Those supplied mainly with nitrate, showed extensive scorch of the margins of medium-aged leaves. This symptom, however, appeared very much later (Figs. 1 & 2). Checks made on the nutrient solutions percolating through the sand showed that the pH was increased in both extreme treatments; the increase being greater with the nitrate treatment than with the ammonium. The same was true of the leachates. As the nutrient solutions are applied daily with pH adjusted to 3.5, the effects of acidity cannot account for the differences in performance of the plants.

When the plants were growing vigorously, in keeping with normal procedure, the concentration of the nutrient solutions was doubled (to Hewitt's solution at normal strength). Soon afterwards, the high ammonium treatments, 1 & 2, showed a distinctive bright yellow border on their younger leaves. As these treatments involve the supply of high levels of sulphur, it was suspected to be a symptom of toxicity of this element or that of the ammonium ion. Accordingly, it was decided to revert to the earlier lower concentration and the symptoms disappeared quickly.

Growth assessments

Comprehensive growth assessments were carried out at the termination of the experiment (at 14 months). The measurements carried out were:

- 1 — Height, total stem length, girth and leaf area,
- 2 — Fresh weights of the entire plants and of stems, leaves and roots separately, and
- 3 — Dry weights of stems, leaves and roots.

Some of the growth measurements are presented in Table 4.



FIG. 1—Leaves of tea plants receiving nitrogen mainly in the ammonium form—
Note diffused chlorosis and necrotic patches

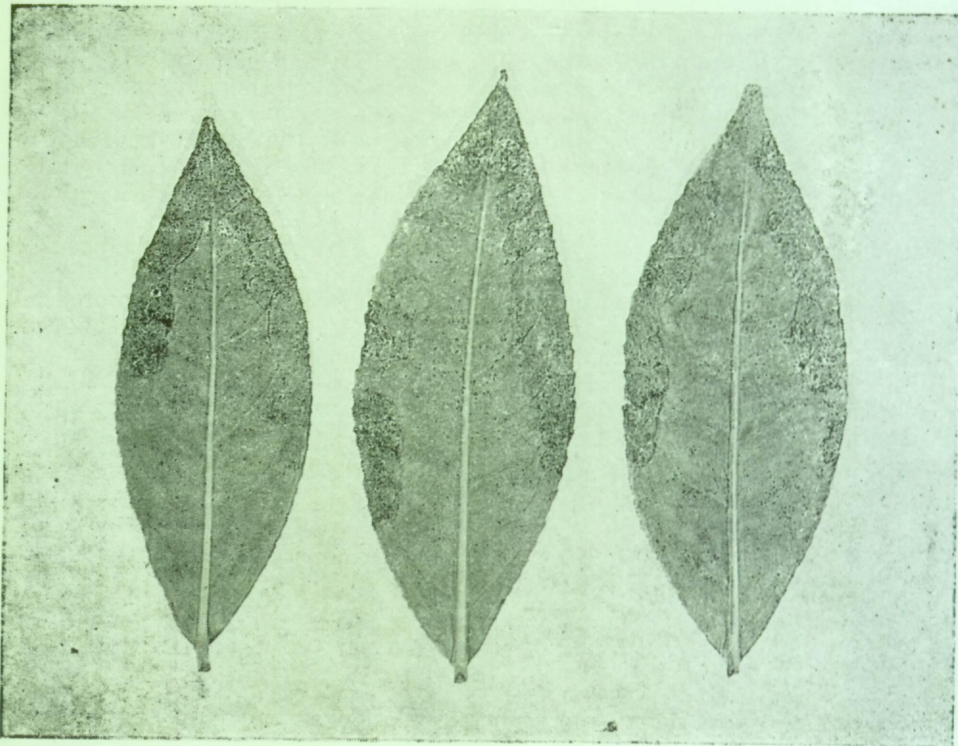


FIG. 2—Leaves of tea plants receiving their nitrogen mainly in the nitrate form, displaying symptoms
of extensive marginal scorch

Chemical analyses

Inorganic, amino, amide and protein nitrogen were estimated in fresh leaves of medium age collected at the time of assessment. This sample comprised the fourth, fifth and sixth leaves of all five replicates of each treatment, bulked together. These represented partially-mature, fully-expanded leaves. Total nitrogen and caffeine nitrogen were determined in dried samples of mature leaves collected at assessment. The fresh leaf samples were macerated in 80% ethanol and ammonium, amide, amino and nitrate nitrogen were determined in the extracts by a modified Kjeldahl method (Barker and Volk 1964). The details of the procedure, are reported in the preceding paper (Kularatne and Bhavanandan 1971). Protein nitrogen was determined on the residue of the ethanol extraction.

Chlorophyll contents of the 80% ethanol extracts combined with acetone extracts of the residue were compared spectrophotometrically. These were found to agree with the observed differences in leaf colour between the treatments. Total free amino acids in the ethanol extracts were also determined by a spectrophotometric method after a ninhydrin colour reaction. The figures obtained confirmed those by the distillation method. The samples of mature leaves that were dried at the time of assessment were used to determine total nitrogen (by the Kjeldahl method), caffeine nitrogen (by a gravimetric method) and calcium and potassium (by flame photometry). The analytical figures for the different nitrogenous fractions are all expressed as mg nitrogen per plant. Tables 5 to 7 present the analytical results.

TABLE 5—*Influence of the form of nitrogen nutrition on the protein, total soluble, caffeine and total nitrogen in the leaves of tea—All figures are expressed as mg N in each fraction per plant*

Treatment	%N as NO ₃	Protein N	Total soluble N	Caffeine N	Total N
1	0	722	106	158	1495
2	10	735	103	112	1295
3	20	980	157	119	1432
4	30	1249	92	146	1861
5	40	1301	130	161	2033
6	50	1614	156	212	2558
7	60	1759	—	271	3072
8	70	1710	—	451	4632
9	80	1528	138	395	4201
10	90	1131	115	395	3322
11	100	935	100	326	2137

(Total N and caffeine N determined on dried samples of mature leaf, Protein and soluble N on fresh samples of younger leaves)

TABLE 6—*Influence of the form of nitrogen nutrition on the various soluble nitrogen fractions in young leaves of tea—All figures expressed as mg N in each fraction per plant*

Treatment	%N as NO ₃	NH ₄ -N	Amide-N	Amino-N	NO ₃ -N	Total
1	0	11.3	18.8	67.5	8.2	105.8
2	10	4.2	26.2	67.0	5.4	102.8
3	20	7.4	32.8	100.5	9.7	157.4
4	30	3.9	19.8	59.2	8.7	91.6
5	40	3.7	30.3	81.8	13.9	129.7
6	50	4.9	39.7	97.2	13.9	155.7
9	80	4.8	27.3	91.4	14.8	138.3
10	90	3.5	22.0	73.6	15.5	114.6
11	100	3.0	22.1	62.3	12.1	99.5

TABLE 7 — *Influence of the form of nitrogen nutrition on the calcium and potassium contents of the leaves of tea— All figures are expressed as a percentage based on dry weight of sample*

Treatment	%N as NO ₃	% Ca	% K
1	0	0.375	1.913
2	10	0.375	1.834
3	20	0.395	1.791
4	30	0.460	1.834
5	40	0.565	1.875
6	50	0.585	1.959
7	60	0.790	2.041
8	70	0.915	2.106
9	80	1.085	2.083
10	90	1.650	2.000
11	100	1.500	2.125

The form of nitrogen supplied over 14 months exerts pronounced effects on the growth of young tea plants and on the nitrogen fractions in the leaves. It is clear from Table 4 that the plants which received 60, 70 and 80% of their nitrogen as nitrate had grown best while those that had received 0, 10 and 20% as nitrate performed poorly. Treatments where nitrate was supplied at 30, 40, 50, 90 and 100% fared only moderately well. It will be noted that there is good agreement in this respect, between all the growth parameters recorded.

If only the two extreme treatments are compared, it is apparent that the plants supplied exclusively with nitrate as the form of nitrogen, have grown better than those supplied only with ammonium nitrogen. This is in agreement with studies on certain other species (*eg* apple, Grassmanis & Leeper 1967 and *Chenopodium*, Kirkby 1967).

The present results are in apparent conflict with the results of field experiments where ammonium fertilizers have generally proved superior to nitrate. Some conversion of ammonia to nitrate must, however, occur within soils. Accordingly under field conditions, even with only ammonium applications, both ionic forms, may be present in the vicinity of absorbing roots. Also, because the nitrate ion is more readily lost by leaching, such fertilizers are likely to appear less efficient. Under our experimental conditions, transformations within the sand are probably minimal. Further, the daily application of nutrient solution would maintain a constant composition of the nutrient mixtures in the root zone and also adequate quantities of them.

In this experiment, the treatments have been imposed over a long period of 14 months and they have resulted in marked effects on growth. This presents a choice in the manner of expression of the analytical figures. The alternatives are to express them as nitrogen contents per unit weight of leaf or as the content per plant. The second basis of expression has been chosen for Tables 5 to 7 and is justified by the wide variations in plant size. In short-term feeding studies, where growth of the plants during the experiment is not great, this complication does not arise and either basis of expression is satisfactory.

Reference to Table 6 indicates that the organic nitrogen and total nitrogen fractions closely follow the growth performance of the plants in the different treatments, the highest levels being generally recovered from the plants of treatments 7, 8 and 9 and the lowest in treatments, 1, 2 and 3. Soluble nitrogen estimates for treatments 7 and 8 had to be excluded from consideration because of an unfortunate breakdown in the functioning of the distillation apparatus.

When the extreme treatments are compared, however, it appears that the often-observed greater utilization of ammonium nitrogen is not evident. That this resulted from a 'dilution effect' due to differential growth between treatments is illustrated by Table 8, where the figures for soluble nitrogenous compounds are expressed as nitrogen contents per 100 g of fresh leaf.

TABLE 8 — *Contents of soluble nitrogenous fractions in samples of young leaves expressed as mg N per 100 g fresh weight of leaf*

Treatment	% N as Nitrate	Ammonium Nitrogen	Amide Nitrogen	Nitrate Nitrogen	Amino Nitrogen	Total
1	0	11.3	18.8	8.2	67.5	105.8
2	10	4.2	26.4	5.5	67.5	103.6
3	20	5.7	30.7	7.5	77.5	121.4
4	30	2.3	11.4	5.0	34.1	52.8
5	40	2.1	17.0	7.8	45.8	72.7
6	50	2.2	17.7	6.2	43.3	69.4
9	80	2.0	11.2	6.1	37.5	56.8
10	90	1.8	11.2	7.9	37.5	58.4
11	100	1.8	13.3	7.3	37.5	59.9

It is apparent that when expressed in this manner, the contents of total soluble nitrogen are greater in the ammonium treatment than in the nitrate. This is particularly so in the amino-nitrogen fraction, in agreement with the well-documented observations in many studies on this subject. The same pattern emerges if total nitrogen contents are considered. The only exception is caffeine nitrogen which is found in greater quantities in the treatments where more nitrate is supplied.

It appears, therefore, that when supplied over a long period, tea makes the best use of a mixture of ammonium and nitrate nitrogen as indicated by the growth of the treated plants. Over a short term, it may well be that ammonium enters more readily and remains mainly in the form of partially elaborated organic compounds (amino acids and amides) and also as the inorganic salts. The disparity in protein nitrogen is not as great between the treatments and, as already stated, caffeine is present in greater quantities in the nitrate-treated plants.

The contents of unmetabolized ammonium and nitrate nitrogen, as expected, generally follow the patterns of supply of the two forms in the nutrient solution. Nitrate is converted to ammonium by plants before elaboration into organic nitrogenous compounds. The presence of relatively high contents of nitrate in plants receiving only ammonium is, however, somewhat puzzling. A probable explanation is that because of imperfections in the method of estimation of nitrate by distillation, this fraction is contaminated with nitrogen from other compounds.

In Table 7 are presented the contents of calcium and potassium in the samples of dried mature leaves. These are expressed as percentages of dry weight. Both cations are present in lesser quantities in the plants in which ammonium is the main form of nitrogen supply; increasing as more of the nitrogen is provided as nitrate. This trend is consistent with competition of the metallic ions with ammonium for entry, as they are all positively charged. It is also noted that the effect is more pronounced with the bivalent calcium ions than with potassium.

It may be mentioned that some leaf symptoms described for the ammonium-fed plants are similar to calcium deficiency symptoms in tea (Pethiyagoda & Krishnapillai 1970) and the levels of calcium in these treatments approach those of deficient plants.

SUMMARY

In respect of both total growth and the contents of elaborated nitrogenous compounds, it is evident that the supply of a mixture of ammonium and nitrate forms of nitrogen is preferred by tea plants. The best performance results when 60-80% of the nitrogen is available in the form of nitrate. In this connexion it may be mentioned that in all our glasshouse experiments, two thirds of the total nitrogen was supplied in this form. The results of this experiment justify this choice.

Ammonium sulphate is the commonest form in which nitrogen is applied to tea. There is, however, ample evidence to show that transformation to the nitrate form occurs in the soil. The conditions promoting an optimal degree of this transformation would clearly have a bearing on response of tea to nitrogen fertilizing. It is to be expected that under the conditions of this experiment, where sand is likely to function as a relatively inert medium, this type of transformation would be minimal.

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