# SAND CULTURE STUDIES OF TEAK (*TECTONA GRANDIS*) IN RELATION TO NUTRITIONAL DEFICIENCY SYMPTOMS, GROWTH AND VIGOUR

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**GOPIKUMAR, K. & VARGHESE, V. 2004. Sand culture studies of teak (*Tectona grandis*) in relation to nutritional deficiency symptoms, growth and vigour.** This study was conducted at the Kerala Agricultural University, India. The objective was to induce symptoms of deficiency of various nutrient elements (N, P, K, Mg, S, Zn and Mo) in seedlings of teak grown in sand culture. The effects of nutrients on the growth, vigour and nutrient uptake pattern were also investigated. Symptoms in seedlings deficient of nutrients include leaf discoloration, necrosis, scorching, defoliation and stunted growth. Seedlings that received complete nutrient solution were healthy and had dark green foliage. Shoot and root growth of the seedlings deficient of nutrients were affected. All the fractions of chlorophyll of treated seedlings, particularly nitrogen-deficient seedlings, reduced considerably. Visual symptoms of nitrogen-deficient seedlings also coincided with the reduction in foliar levels of the concerned element. There was a remarkable improvement in growth of seedlings and the visual symptoms when the deficient element was again supplied to the affected seedlings.

Key words: Chlorophyll – necrosis – discoloration – biomass – antagonism – hormone – fresh – dry weight – defoliation

**GOPIKUMAR, K. & VARGHESE, V. 2004. Kajian kultur pasir bagi jati (*Tectona grandis*) berhubung dengan gejala kekurangan nutrien, pertumbuhan dan kesuburan.** Kajian ini dijalankan di Universiti Pertanian Kerala, India. Tujuannya adalah untuk mengaruh gejala kekurangan beberapa nutrien (N, P, K, Mg, S, Zn dan Mo) dalam anak benih jati yang ditanam dalam kultur pasir. Kesan nutrien terhadap pertumbuhan, kesuburan dan corak pengambilan nutrien juga dikaji. Gejala pada anak benih yang kekurangan nutrien termasuklah pengubahan warna, nekrosis, pelecuran, peranggasan dan pertumbuhan terbantut. Anak benih yang menerima larutan nutrien yang lengkap sihat dan mempunyai daun yang berwarna hijau tua. Pertumbuhan pucuk dan pertumbuhan akar anak benih yang ditanam di dalam larutan kekurangan nutrien terganggu. Kesemua pecahan klorofil dalam anak benih yang dirawat, khasnya anak benih yang kekurangan N, berkurangan dengan banyak. Gejala yang kelihatan pada anak benih kekurangan N berbetulan dengan pengurangan unsur berkenaan dalam daun. Terdapat perbaikan luar biasa dalam pertumbuhan anak benih dan pemulihan gejala yang kelihatan apabila unsur yang berkurangan dibekalkan semula kepada anak benih yang terlibat.

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## Introduction

Teak (*Tectona grandis*) is the principal timber tree of most tropical countries and one of the most important in the world. It is a large deciduous tree with rounded crown and a tall clean cylindrical bole. Despite its immense popularity and commercial importance, the nutritional aspects of teak have seldom been studied, especially in the nursery stage. Severe nutritional disorders have been observed in teak seedlings grown in nurseries. The primary objective of this study was to induce symptoms of deficiency of various nutrient elements in seedlings of teak grown in sand culture. This will enable nursery managers and foresters to use these guidelines for diagnosing nutrient deficiencies of teak in commercial nurseries and plantations. The present study is also aimed at investigating the effect of various nutrient elements on growth, chlorophyll content and uptake pattern of nutrients in the seedling stage.

## Materials and methods

Sand culture experiments to induce the deficiency of nutrients, namely N, P, K, Mg, S, Zn and Mo, in seedlings of teak were carried out at Kerala Agricultural University, India. Thoroughly washed and sterilised quartz silica sand of 250 mesh was used for the sand culture studies.

The studies were conducted inside a glass house under controlled conditions where a mean temperature of 25–30 °C, light intensity of 1200 Lux for a period of 10 hours and a relative humidity of 85% were maintained. Two-month-old healthy seedlings of uniform growth with regard to height, collar girth and leaf number were selected and planted in plastic containers filled with pure quartz sand. All the experimental seedlings were supplied with complete Hoaglands No. 2 (Hoagland 1948) nutrient solution for a period of 10 days until they established well in the sand. After that the following treatments were employed:

Complete Hoaglands nutrient solution (control)
Nutrient solution lacking N
Nutrient solution lacking P
Nutrient solution lacking K
Nutrient solution lacking Mg
Nutrient solution lacking S
Nutrient solution lacking Zn
Nutrient solution lacking Mo

The experiment was laid out in completely randomised design with three replications. The total number of plants for each treatment was 750. The nutrient solutions required for each treatment were carefully prepared in bulk by eliminating the desired nutrient element from the stock. Every alternate day, 50 ml of the nutrient solution were added to each plant. On other days 50 ml of distilled water were added to each plant.

The seedlings were observed daily for symptoms of deficiency. The time taken for the development of various visual symptoms was recorded. Shoot and root growth parameters, fresh and dry matter contents of shoots and roots, chlorophyll content and nutrient elements such as N, P, K, Ca, Mg, S, Zn and Mo were analysed at monthly intervals.

### *Chlorophyll content*

The chlorophyll content of the leaf was estimated spectrophotometrically in a known aliquot of 80% acetone extract. The absorbance was measured at 645 and 663 nm for the estimation of chlorophyll A, chlorophyll B and total chlorophyll. The following formulae suggested by Starner and Hardley (1967) were used for the estimation of different fractions of chlorophyll:

$$\text{Chlorophyll A} = 12.7\,(\text{Abs. at } 663\text{ nm}) - 2.69\,(\text{Abs. at } 645\text{ nm}) \times V/1000 \times W$$
$$\text{Chlorophyll B} = 22.9\,(\text{Abs. at } 645\text{ nm}) - 4.68\,(\text{Abs. at } 663\text{ nm}) \times V/1000 \times W$$
$$\text{Total chlorophyll} = 20.2\,(\text{Abs. at } 645\text{ nm}) + 8.02\,(\text{Abs. at } 663\text{ nm}) \times V/1000 \times W$$

where

Abs. = absorbance
$V$ = final volume of chlorophyll extract (mg)
$W$ = fresh weight of the leaf extract (g)

### *Nutrient element*

Nitrogen was determined at monthly intervals by digesting 0.1 g of the samples in 5 ml concentrated sulphuric acid using hydrogen peroxide and the N in the digest was estimated colorimetrically using Nessler's reagent. The colour was read in a spectrophotometer at 410 nm. Phosphorus was determined in a known aliquot of the acid extract colorimetrically by the Vanado-molybdophosphoric yellow colour method (Jackson 1958). The yellow colour was read in a spectrophotometer at a wave length of 470 nm. Potassium was estimated in a known volume of the acid extract using a flame photometer. Calcium, Mg, Zn and Mo were estimated in Atomic Absorption spectrophotometer using respective hollow cathode tubes. Sulphur was estimated turbidometrically at 400 nm using diacid extract in the presence of barium chloride (Jackson 1958)

### *Recovery studies*

At the end of six months, after imposing various treatments, representative seedlings from each replication showing nutrient deficiency symptoms were selected for the recovery studies. The nutrient element which was deficient earlier was supplied through complete Hoaglands solution. The improvement in growth of the seedlings and recovery of leaf discoloration were noticed. All the observations made earlier were repeated here too. At the end of the recovery studies seedlings were also analysed for various nutrient elements.

## Results

### *Visual deficiency symptoms*

The seedlings that received all the nutrients through complete Hoaglands nutrient solution were found to be very vigorous and healthy in growth and produced dark green, normal-shaped foliage throughout the study period. These seedlings did not show any visual symptoms of deficiency.

The symptoms of N deficiency appeared at the end of the first month. During the initial stages, yellow patches appeared towards the margins of older leaves. Later the entire lamina turned pale yellow. Stunting of the seedlings was also noticed at this stage. In the acute stage of deficiency, the entire seedling appeared severely chlorotic and these leaves gradually dried prematurely.

Symptoms of P deficiency appeared about two months after treatment. Symptoms appeared first on older leaves as purple bronze patches which later changed into yellow chlorotic patches. New leaves were pale in colour. Gradually the bronze patches extended towards the entire leaf resulting in premature defoliation. At the end of the study, seedlings had sparse foliage and were stunted in growth compared with the control.

Deficiency symptoms of K started appearing in the third month. The symptoms were first manifested on lower leaves. The leaves had chlorotic tips at the beginning. These chlorotic areas gradually spread through the margin upwards. Necrosis progressed from the lower part of chlorotic leaves. This stage was noticed during the fifth month.

Magnesium deficient symptoms were noticed from the 50th day onwards. The older leaves produced small necrotic areas during the initial stages of deficiency. Characteristic chlorotic pattern between the veins was noticed. However, the midrib and the veins remained green. At the acute stages, the chlorotic areas developed into necrotic regions. The seedlings were also stunted in growth.

Sulphur deficiency symptoms first appeared after three months as discoloration of the terminal leaves from dark green to pale green. The symptoms gradually advanced from the margin inwards. At the moderate stages of deficiency, only the regions close to the midrib appeared green. Later, necrosis set in and at the acute stage the entire leaf developed chlorotic. The affected leaves were yellowish in colour.

Zinc deficiency symptoms were noticed from the fourth month onwards. The symptoms first appeared on the lower leaves as chlorotic patches. Seedlings were stunted in growth with short internodes, more number of branches and small clustered leaves. Later the leaves developed necrotic patches and at the severe stages the leaves had a burnt appearance.

Seedlings which lacked Mo developed deficiency symptoms after five months. The symptoms first appeared on terminal leaves. The size of terminal leaves reduced considerably. The leaves were narrow in appearance. At later stages interveinal chlorosis was noticed.

### *Shoot growth parameters*

At the end of the study period, seedlings grown in complete nutrient solution had a maximum height growth of 53.0 cm while the N-deficient seedlings recorded the lowest height growth of 17.5 cm (Table 1). Among the various nutrients, Mo produced maximum height growth (45.7 cm) at the end of the study period, which was only 13.8% less compared with the control. This was followed by Zn- and K-deficient seedlings. Seedlings that were S deficient were on a par with those deficient in Mg in terms of height at the end of the study period. Height increment was relatively less in all the treatments compared with the control.

With regard to collar diameter, N-deficient seedlings recorded 51.2% lower value compared with the control. In the case of Mo-deficient seedlings, it was only 11.6% lower compared with the control. For P and K, the mean diameter was each 0.29 cm at the end of the study. The mean collar diameter for Zn-deficient seedlings was 0.3 cm at the end of the study.

The seedlings receiving complete nutrient solution produced the highest number of leaves (26) at the end of the study period (Table 2). At the end of the study, the N-deficient seedlings had a leaf number of 10. This was followed by P-deficient (14) and Mg-deficient (16 ) seedlings. The S- and Zn-deficient seedlings produced a leaf number of 20 and 21 respectively at the end of the study.

Maximum leaf area was recorded by seedlings grown in complete nutrient solution (Table 2). It showed an increasing trend from 1506.5 $cm^2$ at the commencement of the treatment to 3240.7 $cm^2$ at the end of the study. The lowest leaf area (520.7$cm^2$) was recorded by seedlings lacking N. Throughout the study period, it never recorded beyond 600.0 $cm^2$. The second lowest in leaf area were seedlings which were deficient in P. At the end of the study, it recorded an area of 935.7 $cm^2$. In the case of S- and Mo-deficient plants, the leaf areas were 2227.0 and 2933.8 $cm^2$ respectively at the end of the study. The difference in leaf area between Mg- and Zn-deficient seedlings was not significant.

### *Fresh and dry weights of shoots*

With regard to fresh weight of shoots, treatment differences were very pronounced from the second month onwards (Table 3). Seedlings that received complete nutrient solution recorded the highest shoot fresh weight from the beginning of the study. Among the nutrient elements, Mo-deficient seedlings recorded the highest shoot fresh weights of 37.0 and 45.7 g respectively during the fifth and sixth month. Except for the first month, the N-deficient plants recorded the lowest shoot fresh weight throughout the study. During the second month, it was lowest (7.0 g) and at the end of the study period, the plants recorded a fresh weight of 11.4 g. The shoot fresh weight of the rest of the treatments were in the order K < Mg < P < S < Zn < Mo < Control.

**Table 1** Effect of nutrient deficiencies on shoot growth parameters of seedlings

| Nutrient element removed from complete solution | Month 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Height (cm) | Collar diameter (cm) | Height (cm) | Collar diameter (cm) | Height (cm) | Collar diameter (cm) | Height (cm) | Collar diameter (cm) | Height (cm) | Collar diameter (cm) | Height (cm) | Collar diameter (cm) |
| N | 13.38 | 0.14 | 15.12 | 0.17 | 16.28 | 0.17 | 16.31 | 0.19 | 16.50 | 0.21 | 17.49 | 0.21 |
| P | 14.04 | 0.14 | 18.00 | 0.19 | 25.77 | 0.21 | 27.29 | 0.24 | 31.47 | 0.25 | 34.55 | 0.29 |
| K | 14.70 | 0.15 | 21.24 | 0.19 | 27.12 | 0.23 | 28.66 | 0.25 | 33.21 | 0.26 | 40.34 | 0.29 |
| Mg | 13.59 | 0.16 | 20.51 | 0.21 | 25.73 | 0.25 | 29.14 | 0.27 | 32.88 | 0 30 | 37.41 | 0.31 |
| S | 15.46 | 0.14 | 22.32 | 0.18 | 25.60 | 0.21 | 29.59 | 0.21 | 32.32 | 0.24 | 38.09 | 0.26 |
| Zn | 16.43 | 0.16 | 20.68 | 0.20 | 27.30 | 0.24 | 33.51 | 0.27 | 35.68 | 0.31 | 40.74 | 0.34 |
| Mo | 16.02 | 0.16 | 20.29 | 0.19 | 30.68 | 0.23 | 39.86 | 0.28 | 43.52 | 0.32 | 45.72 | 0.38 |
| Control | 17.76 | 0.18 | 24.71 | 0.22 | 34.90 | 0.26 | 43.85 | 0.33 | 49.48 | 0.36 | 53.03 | 0.43 |
| F-test | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| SEM | 0.34 | 0.00 | 0.36 | 0.00 | 0.39 | 0.00 | 0.45 | 0.00 | 0.39 | 0.00 | 0.56 | 0.01 |
| CD (5%) | 1.03 | 0.08 | 1.09 | 0.05 | 1.18 | 0 05 | 1.34 | 0.08 | 1.16 | 0.05 | 1.67 | 0.05 |

** significant at the 0.01 probablity level

**Table 2** Effect of nutrient deficiencies on leaf production of seedlings

| Nutrient element removed from complete solution | Month 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of leaves | Leaf area ($cm^2$) | No. of leaves | Leaf area ($cm^2$) | No. of leaves | Leaf area ($cm^2$) | No. of leaves | Leaf area ($cm^2$) | No. of leaves | Leaf area ($cm^2$) | No. of leaves | Leaf area ($cm^2$) |
| N | 10 | 576.27 | 12 | 547.56 | 12 | 559.11 | 12 | 534.00 | 11 | 550.11 | 10 | 520.67 |
| P | 10 | 763.16 | 11 | 879.30 | 14 | 1255.67 | 14 | 1744.87 | 14 | 1044.22 | 14 | 935.69 |
| K | 11 | 868.27 | 14 | 1625.46 | 18 | 1905.90 | 18 | 1606.56 | 20 | 1806.28 | 19 | 1339.90 |
| Mg | 11 | 872.15 | 13 | 1979.50 | 16 | 1731.56 | 17 | 1554.26 | 17 | 1417.56 | 16 | 1058.39 |
| S | 11 | 620.31 | 14 | 1600.25 | 16 | 1605.53 | 17 | 1332.78 | 20 | 1502.22 | 20 | 2227.00 |
| Zn | 11 | 1048.35 | 13 | 1248.00 | 17 | 1343.22 | 18 | 1266.47 | 17 | 1563.26 | 21 | 1080.63 |
| Mo | 11 | 1039.46 | 14 | 1339.69 | 15 | 1762.56 | 18 | 2160.44 | 20 | 2384.71 | 22 | 2933.78 |
| Control | 15 | 1506.45 | 17 | 1983.05 | 19 | 2048.78 | 22 | 2057.66 | 23 | 2461.11 | 26 | 3240.68 |
| F-test | ns | * | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| SEM | 0.27 | 135.68 | 0.32 | 108.00 | 0.32 | 139.63 | 0.35 | 167.39 | 0.35 | 169.54 | 0.48 | 88.65 |
| CD (5%) | - | 406.8 | 0.92 | 323.8 | 0.94 | 418.6 | 1.08 | 501.8 | 0.99 | 508.3 | 1.39 | 265.80 |

ns = not significant, ** = significant at the 0.01 probability level, * = significant at the 0.01 probability level

Dry weight of the shoots produced by different nutrient treatments also differed significantly from the second month onwards (Table 3). With regard to dry weight, the treatments were in the order N < P < Mg < K < S < Zn < Mo < Control.

### *Root growth parameters*

The length of the main root did not show any significant difference due to the treatment except for the last month (Table 4). At the end of the study, K-deficient seedlings recorded the lowest root length (16.8 cm) compared with the control (26.7 cm). Zinc-deficient seedlings recorded maximum root length (24.4 cm) at the end of the study. At this period, N- and P-deficient seedlings recorded root lengths of 21.0 and 20.6 cm respectively. Magnesium-deficient seedlings produced roots of 19.2 cm long at the end of the study.

Except at the end of the sixth month, nutrient-deficient treatments did not bring about any significant difference in the number of secondary roots produced by the seedlings (Table 4). Magnesium-deficient seedlings had the lowest number of secondary roots in the first and last month (29 each) of the study. The S- and N-deficient seedlings had the second lowest number of secondary roots (32) at the end of the study. In general, Mo- and Zn-deficient seedlings produced greater number of secondary roots throughout the study period. However, their numbers were slightly lower than that of seedlings grown in complete nutrient solution.

### *Fresh and dry weights of roots*

Root fresh and dry weights were influenced by the different treatments from the second month onwards (Table 5). Seedlings grown in complete nutrient solution recorded the highest fresh weight of 18.7 g at the sixth month. Zinc-deficient seedlings showed the highest root fresh weight at the sixth month (15.3 g), followed by Mo-deficient seedlings (15.2 g). At the end of the study, fresh weight of roots of N-deficient plants was the lowest (10.7 g) followed by K-deficient plants (12.4 g).

The root dry weight at the end of the study were in the order K < N < Mg < S < P < Zn < Mo < Control. At the end of the study, the root dry weight of the seedlings grown in complete nutrient solution was 7.4 g, while it was only 5.3 g in K-deficient seedlings.

### *Chlorophyll content*

The chlorophyll content of the leaves was significantly influenced by the deficiency of various nutrient elements (Table 6). The N-deficient seedlings had the lowest chlorophyll-A content (0.32 mg $g^{-1}$) followed by S-deficient seedlings (0.33 mg $g^{-1}$) at the end of the study period. The seedlings receiving complete nutrient solution had higher contents of all fractions of chlorophyll. Chlorophyll-B content also declined gradually for all the treatments except for K. The N-deficient seedlings recorded the lowest content of chlorophyll B at the end of the study

**Table 3** Effect of nutrient deficiencies on the fresh and dry weights(g) of shoots

| Nutrient element removed from complete solution | Month 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight |
| N | 12.01 | 3.50 | 7.01 | 2.91 | 11.39 | 3.70 | 11.23 | 4.01 | 10.87 | 3.93 | 11.39 | 4.19 |
| P | 10.63 | 3.05 | 12.82 | 4.23 | 23.85 | 7.58 | 31.51 | 11.75 | 27.48 | 11.57 | 33.69 | 10.17 |
| K | 12.98 | 3.71 | 12.35 | 3.63 | 17.37 | 6.64 | 23.42 | 10.84 | 27.45 | 8.58 | 31.61 | 11.31 |
| Mg | 13.43 | 3.98 | 18.82 | 6.61 | 21.98 | 7.13 | 21.72 | 9.54 | 29.03 | 11.60 | 32.61 | 10.67 |
| S | 12.91 | 3.93 | 10.57 | 3.08 | 18.79 | 8.82 | 27.00 | 11.84 | 29.06 | 10.03 | 34.88 | 11.55 |
| Zn | 12.55 | 3.84 | 14.31 | 4.35 | 21.02 | 9.44 | 25.20 | 10.92 | 31.21 | 11.26 | 35.35 | 11.61 |
| Mo | 12.61 | 3.73 | 16.24 | 5.53 | 25.22 | 8.47 | 36.03 | 9.37 | 36.95 | 11.71 | 45.72 | 15.30 |
| Control | 14.20 | 4.04 | 20.99 | 7.40 | 35.03 | 10.58 | 47.12 | 12.96 | 50.60 | 14.38 | 65.84 | 18.23 |
| F-test | ns | ns | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| SEM | 0.68 | 0.26 | 0.51 | 0.39 | 0.99 | 0.41 | 0.66 | 0.44 | 0.91 | 0.61 | 0.92 | 1.07 |
| CD (5%) | – | – | 1.54 | 1.17 | 2.96 | 1.25 | 1.97 | 1.31 | 2.73 | 1.84 | 2.77 | 3.20 |

ns = not significant, ** = significant at the 0.01 probablity level

**Table 4** Effect of nutrient deficiencies on root growth parameters of seedlings

| Nutrient element removed from complete solution | Month 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length of root (cm) | No. of secondary roots | Length of root (cm) | No. of secondary roots | Length of root (cm) | No. of secondary roots | Length of root (cm) | No. of secondary roots | Length of root (cm) | No. of secondary roots | Length of root (cm) | No.of secondary roots |
| N | 20.76 | 30 | 22.08 | 42 | 21.23 | 35 | 22.40 | 36 | 20.33 | 34 | 20.97 | 32 |
| P | 19.33 | 32 | 22.84 | 39 | 21.18 | 36 | 22.30 | 35 | 21.67 | 43 | 20.61 | 33 |
| K | 19.23 | 31 | 23.74 | 41 | 20.94 | 38 | 22.51 | 34 | 21.80 | 37 | 16.79 | 38 |
| Mg | 20.60 | 29 | 22.09 | 35 | 20.52 | 34 | 21.82 | 35 | 20.63 | 34 | 19.20 | 29 |
| S | 19.98 | 30 | 22.08 | 40 | 20.84 | 33 | 21.67 | 33 | 21.35 | 36 | 22.20 | 32 |
| Zn | 19.81 | 32 | 22.36 | 37 | 20.18 | 34 | 22.65 | 34 | 20.73 | 35 | 24.35 | 42 |
| Mo | 20.06 | 33 | 22.51 | 35 | 20.73 | 39 | 22.55 | 40 | 21.83 | 42 | 20.18 | 41 |
| Control | 29.13 | 53 | 25.13 | 47 | 24.73 | 43 | 26.18 | 43 | 24.90 | 44 | 26.67 | 43 |
| F-test | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | * | * |
| SEM | 2.09 | 2.11 | 1.78 | 1.96 | 1.21 | 2.35 | 1.48 | 2.64 | 1.28 | 2.16 | 1.78 | 3.13 |
| CD (5%) | – | – | – | – | – | – | – | – | – | – | 5.28 | 9.40 |

ns = not significant, ** = significant at the 0.01 probability level, * = significant at the 0.05 probability level

period (0.3 mg $g^{-1}$). Similarly, seedlings subjected to N stress recorded the lowest total chlorophyll content throughout the study period. Here the total chlorophyll content was found to decrease from 2.4 mg $g^{-1}$ on the second month to 0.6 mg $g^{-1}$ on the last month.

### *Tissue nutrient levels*

The N content of leaves decreased in seedlings supplied with nutrient solution lacking N (Table 7). In these seedlings, N content gradually decreased from 1.0% in the beginning to 0.6% by the end of the sixth month. The seedlings receiving complete nutrient solution recorded highest N content (1.5%). Phosphorus-deficient seedlings had N concentration of 1.34%, which was the second highest. In the case of Mg- and S-deficient seedlings, moderate contents of 1.23 and 1.24% N were recorded.

The tissue concentration of P in plants supplied with nutrient solutions lacking this element showed a gradual decline during the course of the study. At the end of the study, P concentration in these plants decreased to 0.4% from the initial content of 1.3%. At the end of the study, the seedlings receiving complete nutrient solution recorded the highest value of 1.4%. All the other nutrient-deficient treatments recorded lower levels of P during the course of the study.

All the treatments were statistically significant with regard to their K content. The seedlings supplied with nutrient solution lacking K showed a decreasing tendency from the initial content of 1.3% to 0.3%. At the end of the study, S-deficient treatment recorded the highest value of 1.54%, followed by seedlings receiving complete nutrient solution (1.51%). The seedlings which were deficient in N showed a decreasing tendency with regard to the K content. In the case of P-, Mg- and Mo-deficient treatments, the seedlings recorded the K content of 1.37, 1.33 and 1.30% respectively at the end of the study period.

The seedlings grown using solutions deficient in K and Mo recorded more or less similar values with regard to Ca content, while the seedlings deficient in N, P, Mg and Zn showed a decreasing tendency as the study progressed. At the end of the study, the seedlings receiving complete nutrient solution had the highest (1.2%) and those deficient in Zn had the lowest content (0.8%).

In seedlings supplied with nutrient solution lacking Mg, the concentration of Mg fell from an initial level of 1.3 to 0.7% at the end of the study. However, in the case of seedlings grown with nutrient solution lacking N, there was not much variation in the content of Mg throughout the study period. With regard to most of the other treatments, the Mg content showed a decreasing tendency with the progress of the study. In the case of seedlings receiving complete nutrient solution, the Mg content increased slightly at the end of the study.

The concentration of S content was lowest in seedlings supplied with solution lacking S. The initial content of S was 0.04% and at the end of the study, it was 0.02%. The reduction from initial value to final value was 50%. In all the other nutrient-deficient seedlings, there was not much fluctuation in the content of S from beginning to end. At the end of the study, the highest value was recorded by seedlings which were receiving complete nutrient solution (0.06%) .

**Table 5** Effect of nutrient deficiencies on the fresh and dry weights (g) of roots

| Nutrient element removed from complete solution | Month 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight | Fresh weight | Dry weight |
| N | 4.12 | 1.74 | 13.29 | 4.53 | 7.67 | 2.83 | 8.19 | 4.22 | 11.26 | 4.93 | 10.73 | 5.37 |
| P | 4.18 | 1.87 | 6.23 | 2.05 | 7.99 | 3.01 | 10.90 | 5.19 | 12.17 | 4.80 | 12.87 | 5.73 |
| K | 4.19 | 1.82 | 8.11 | 2.52 | 11.30 | 4.32 | 10.79 | 4.42 | 10.70 | 4.27 | 12.39 | 5.33 |
| Mg | 4.13 | 1.85 | 7.50 | 2.58 | 8.28 | 3.79 | 10.62 | 5.21 | 12.73 | 5.41 | 13.44 | 5.47 |
| S | 4.53 | 1.66 | 13.79 | 3.14 | 9.18 | 3.99 | 9.01 | 3.93 | 9.87 | 4.43 | 14.70 | 5.63 |
| Zn | 4.47 | 1.54 | 14.78 | 3.17 | 10.06 | 3.78 | 10.40 | 4.42 | 13.52 | 5.38 | 15.32 | 6.32 |
| Mo | 4.89 | 1.93 | 11.95 | 2.90 | 7.54 | 2.55 | 13.36 | 5.62 | 14.92 | 6.75 | 15.16 | 6.79 |
| Control | 4.98 | 1.76 | 10.78 | 2.44 | 13.24 | 3.98 | 15.57 | 7.19 | 16.26 | 7.54 | 18.68 | 7.43 |
| F-test | ns | ns | ** | ** | ** | ** | ** | ** | ** | ** | ** | * |
| SEM | 0.23 | 0.11 | 0.68 | 0.23 | 0.35 | 0.31 | 0.71 | 0.40 | 0.68 | 0.50 | 0.73 | 0.44 |
| CD (5%) | – | – | 2.03 | 0.68 | 1.05 | 0.94 | 2.14 | 1.20 | 2.05 | 1.51 | 2.20 | 1.31 |

ns = not significant, ** = significant at the 0.01 probability level, * = significant at the 0.05 probability level

**Table 6** Effect of nutrient deficiencies on the chlorophyll content of leaf tissue of seedlings

| Nutrient element removed from complete solution | Month 2 | 3 | 4 | 5 | 6 | 2 | 3 | 4 | 5 | 6 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chlorophyll A (mg $g^{-1}$) | | | | | Chlorophyll B (mg $g^{-1}$) | | | | | Total chlorophyll (mg $g^{-1}$) | | | | |
| N | 0.89 | 0.72 | 0.63 | 0.42 | 0.32 | 1.50 | 0.69 | 0.65 | 0.44 | 0.31 | 2.39 | 1.41 | 1.28 | 0.86 | 0.63 |
| P | 1.97 | 1.29 | 0.60 | 0.56 | 0.61 | 1.75 | 1.28 | 1.14 | 0.84 | 0.73 | 3.72 | 2.57 | 1.74 | 1.40 | 1.34 |
| K | 1.59 | 1.29 | 1.17 | 0.69 | 0.58 | 1.26 | 1.35 | 1.24 | 1.11 | 1.03 | 2.85 | 2.64 | 2.41 | 1.80 | 1.61 |
| Mg | 2.07 | 1.60 | 0.88 | 0.68 | 0.71 | 1.45 | 1.19 | 1.16 | 1.11 | 0.93 | 3.53 | 2.80 | 2.04 | 1.79 | 1.64 |
| S | 2.06 | 1.10 | 0.91 | 0.64 | 0.33 | 1.71 | 1.38 | 1.14 | 0.90 | 0.74 | 3.77 | 2.48 | 2.05 | 1.54 | 1.07 |
| Zn | 2.11 | 1.31 | 0.78 | 0.57 | 0.41 | 1.24 | 1.10 | 0.95 | 0.84 | 0.67 | 3.23 | 2.41 | 1.74 | 1.42 | 1.08 |
| Mo | 1.81 | 1.29 | 0.91 | 0.80 | 0.62 | 1.32 | 1.09 | 0.83 | 0.81 | 0.67 | 3.13 | 2.38 | 1.74 | 1.61 | 1.29 |
| Control | 2.09 | 1.65 | 1.39 | 0.80 | 0.58 | 1.60 | 1.14 | 0.99 | 0.97 | 0.87 | 3.69 | 2.78 | 2.38 | 1.77 | 1.44 |
| F-test | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| SEM | 0.05 | 0.03 | 0.06 | 0.03 | 0.04 | 0.09 | 0.03 | 0.04 | 0.03 | 0.03 | 0.09 | 0.03 | 0.05 | 0.04 | 0.04 |
| CD (5%) | 0.14 | 0.08 | 0.17 | 0.09 | 0.11 | 0.26 | 0.09 | 0.12 | 0.09 | 0.08 | 0.27 | 0.09 | 0.14 | 0.11 | 0.11 |

**significant at the 0.01 probability level

The seedlings which were deficient in Zn recorded the lowest value at the end of the study (0.004%). Similarly, in most of the nutrient treatments, the Mo content showed a decreasing tendency with the progress of the study.

*Recovery studies using complete nutrient solution*

During the course of recovery studies, the foliar symptoms such as leaf discoloration induced by the deficiency of various nutrient elements gradually disappeared. The new flushes of the leaves produced were healthy, green and had normal shape. Height increase was significant after the commencement of the recovery studies.

The N-deficient seedlings had height increment from 16.3 to 23.2 cm, while P-deficient seedlings recorded an increment of 27.3 to 38.3 cm (Table 8). Diameter increase was also significant during the recovery studies. This was more pronounced for seedlings deficient in Zn and P. The increments were from 0.20 to 0.36 cm and 0.24 to 0.33 cm respectively.

The chemical analysis of the leaf tissues at the recovery studies revealed that there was improvement in the concentration of major nutrients in the seedlings when compared with the nutrient content before the recovery studies. In the case of N-deficient seedlings, the N content increased from 0.60 to 1.34% on receiving complete nutrient solution. With regard to P treatment, there was an improvement from 0.42 to 1.22% in P concentration. Similarly, the K content of K-deficient seedlings before recovery studies was 0.3% and had been increased to 1.5% after the recovery studies. The application of complete nutrient solution had increased the content of Mg, S, Zn and Mo in seedlings which were formerly deficient in these elements.

## Discussion

The present study was conducted with an objective of inducing and describing the symptoms of deficiency of various nutrient elements in the seedlings. This will also provide information on understanding the importance of nutrient elements, their actual role, quantity required and uptake pattern which will finally benefit the foresters and farmers for the production of healthy and vigorous seedlings for extensive planting programmes.

The initial symptom of nitrogen deficiency was the development of yellow chlorotic patches in the older leaves. The acute stage of nitrogen deficiency was characterised by severe chlorosis of the entire seedling followed by premature drying and defoliation. Chlorophyll content was also found to decline gradually in these seedlings. Chlorosis of the older leaves was a result of inadequate supply of N for chloroplast protein synthesis. Nitrogen deficiency has pronounced effect on the growth behaviour of seedlings particularly with regard to shoot growth. Similar observations were also made by Kaul *et al.* (1972) in teak and Anoop (1993) in *Ailanthus*. The reduction in vegetative growth may be due to the fact that N

**Table 7** Effect of nutrient deficiencies on tissue nutrient levels

| Nutrient element removed from complete solution | N% | | P% | | K% | | Ca% | | Mg% | | S% | | Zn% | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| N | 1.02 | 0.56 | 1.28 | 1.21 | 1.78 | 0.98 | 0.94 | 0.93 | 0.78 | 0.94 | 0.038 | 0.037 | 0.013 | 0.008 |
| P | 1.71 | 1.34 | 1.26 | 0.42 | 1.94 | 1.37 | 1.24 | 0.92 | 1.79 | 0.90 | 0.047 | 0.037 | 0.013 | 0.016 |
| K | 1.47 | 1.08 | 1.98 | 1.13 | 1.31 | 0.28 | 1.27 | 1.16 | 1.34 | 1.18 | 0.048 | 0.046 | 0.027 | 0.009 |
| Mg | 1.62 | 1.23 | 1.52 | 1.23 | 1.96 | 1.33 | 1.28 | 0.90 | 1.27 | 0.70 | 0.042 | 0.036 | 0.020 | 0.006 |
| S | 1.59 | 1.24 | 1.99 | 1.15 | 1.72 | 1.54 | 1.04 | 1.01 | 1.91 | 0.97 | 0.042 | 0.020 | 0.013 | 0.013 |
| Zn | 1.50 | 1.14 | 1.89 | 1.09 | 1.78 | 1.47 | 1.12 | 0.79 | 1.23 | 1.10 | 0.040 | 0.034 | 0.013 | 0.004 |
| Mo | 1.32 | 1.30 | 2.07 | 1.20 | 1.80 | 1.30 | 1.17 | 1.11 | 1.13 | 1.63 | 0.052 | 0.044 | 0.017 | 0.009 |
| Control | 1.42 | 1.53 | 2.48 | 1.39 | 1.82 | 1.51 | 1.41 | 1.22 | 2.57 | 2.59 | 0.047 | 0.058 | 0.033 | 0.013 |
| F-test | * | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| SEM | 0.11 | 0.08 | 0.07 | 0.06 | 0.09 | 0.10 | 0.07 | 0.04 | 0.03 | 0.04 | 0.01 | 0.01 | 0.01 | 0.01 |
| CD (5%) | 0.34 | 0.25 | 0.20 | 0.18 | 0.26 | 0.31 | 0.23 | 0.11 | 0.08 | 0.11 | 0.05 | 0.05 | 0.05 | 0.05 |

* = significant at the 0.05 probability level, ** = significant at 0.01 probability level

**Table 8** Final growth parameters and tissue nutrient content after the application of complete solution for recovery

| Nutrient element removed from complete solution | Height (cm) | Diameter (cm) | Leaf no. | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | Zn (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| N | 23.20 | 0.22 | 14.18 | 1.34 | 1.93 | 2.17 | 1.04 | 1.95 | 0.057 | 0.020 |
| P | 38.34 | 0.33 | 19.33 | 1.51 | 1.22 | 1.73 | 0.98 | 1.19 | 0.055 | 0.011 |
| K | 40.42 | 0.33 | 19.04 | 1.40 | 2.01 | 1.53 | 1.39 | 1.57 | 0.063 | 0.016 |
| Mg | 36.92 | 0.31 | 20.17 | 1.40 | 1.48 | 1.30 | 1.32 | 1.86 | 0.067 | 0.024 |
| S | 39.28 | 0.33 | 21.09 | 1.23 | 2.27 | 1.75 | 1.21 | 1.94 | 0.050 | 0.032 |
| Zn | 40.19 | 0.36 | 20.74 | 1.34 | 2.33 | 1.33 | 1.16 | 1.85 | 0.052 | 0.010 |
| Mo | 49.28 | 0.36 | 24.76 | 1.29 | 2.30 | 1.55 | 1.12 | 1.29 | 0.051 | 0.026 |
| Control | 53.35 | 0.40 | 24.41 | 1.68 | 2.34 | 1.98 | 1.34 | 1.53 | 0.059 | 0.035 |
| F-test | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| SEM | 1.65 | 0.01 | 1.21 | 0.11 | 0.07 | 0.13 | 0.06 | 0.04 | 0.01 | 0.01 |
| CD (5%) | 4.94 | 0.05 | 3.64 | 0.34 | 0.21 | 0.38 | 0.16 | 0.12 | 0.05 | 0.05 |

** = significant at the 0.01 probability level

largely controls the use of carbohydrates and hence determines whether the plant will make vegetative or reproductive growth (Jones & Embleton 1959). With regard to tissue concentration, by the end of the study, N concentration fell to 0.6% from an initial content of 1.0%. This coincided with the severe stage of deficiency when the entire seedling appeared chlorotic followed by premature drying and defoliation. Deletion of N from the treatment solution increased P concentration (1.2%) of these seedlings at the end of six months. Antagonistic effect of N and P has been reported in cocoa (Lockard & Asomaning 1964) and pepper (Nybe 1986). The K concentration of N-deficient seedlings recorded a decreasing tendency. Interestingly the N-deficient plants recovered from the visual symptoms of deficiency and produced green foliage by the end of the recovery studies. There was also a rapid improvement in height growth but improvement in collar diameter was relatively low. The foliar concentration was also found to increase remarkably with the application of complete solution. Landis *et al.* (1989) noted that in paper birch stunting due to N deficiency was usually easy to diagnose and subsequently easy to correct because deficient seedlings rapidly respond to application of N fertilisers.

Phosphorus-deficiency symptoms appeared first on older leaves as purple bronze patches. As deficiency advanced, these purple bronze patches extended to the entire leaves and later changed to yellow chlorotic patches. Phosphorus deficiency is reported to result in the formation and accumulation of anthocyanin pigments, which lead to the development of purple coloration (Landis *et al.* 1989). Shoot growth parameters such as height, girth and leaf production were significantly affected by P deficiency. The sand culture studies conducted by Gopikumar and Aravindakshan (1988) in cashew and Anoop (1993) in *Ailanthus* also revealed similar result for P deficiency. Shoot fresh and dry weights also recorded lower leaves here. This may be due to the fact that like N, P also plays an important role as the structural component of the cell constituents and other metabolically active compounds (Greulach 1973) and is the major controlling factor of energy in all living cells. In P-deficient seedlings, the concentration of P in the tissues decreased gradually as visual deficiency symptoms progressed. Phosphorus deficiency caused a decrease in the foliar levels of N, K, Ca and Mg. Similar results were also reported in apples (Landis *et al.* 1989), nutmeg (Philip 1986) and teak (Kaul *et al.* 1972). In P-deficient seedlings, the extent of recovery of visual symptoms was remarkable on application of the complete nutrient solution. There was an improvement in all growth parameters. It is also interesting to note that the P content in these seedlings increased at the end of the recovery studies and attained a concentration similar to healthy seedlings, thereby indicating the possibilities of improving seedling growth and control of the deficiency by its application.

The K-deficient seedlings showed chlorotic tips in the older leaves by the third month. Necrosis of the leaf lamina at the acute stage of deficiency might have resulted from the accumulation of diamine and putriscine as reported by Drechsel and Zech (1991). Even though there was a gradual decline in chlorophyll B and total chlorophyll of K-deficient seedlings, they generally had a higher concentration compared with other treatments particularly at the end of the study. Though K

activated the synthesis of chlorophyll, an increased partitioning of K to the chloroplast has been reported as the reason for no substantial reduction in chlorophyll content and photosynthetic rates in K-deficient plants (Capron *et al.* 1982). In the present study, reductions in height, collar diameter and leaf production were not found to vary significantly in K-deficient seedlings as compared with other treatments. Potassium deficiency was associated with a decrease in the initial foliar content of K from 1.3 to 0.3% at the end of the study. The data revealed that the content of N, P, Mg and S decreased on account of K deficiency. Among all the interactions, the antagonistic effect of K with Mg was most pronounced which has also been reported by Nybe (1986) in pepper and Anoop (1993) in *Ailanthus*. On application of complete nutrient solution, seedlings recovered well from the visual symptoms and growth retardation induced by K deficiency.

Magnesium deficiency produced typical visual symptoms as interveinal chlorosis with reticulate pattern. In acute stage, these chlorotic patches between the green midrib and the veins developed necrosis. Growth was stunted in these seedlings compared with the control. In nutmeg, Philip (1986) also noted similar symptoms. Magnesium is known to play a catalytic role as activator of a number of enzymes, most of which are concerned with carbohydrate metabolism, phosphate transfer and decarboxylation (Dixon 1949). The development of chlorosis between the veins of the leaves occurred when the Mg content of the tissue declined to lower levels. Antagonistic effect of Mg with all other elements except P was evident from the present study. Antagonistic influence of Mg with K and Ca had been reported by Nybe (1986) in pepper and Anoop (1993) in *Ailanthus*. Magnesium-deficient plants recovered well by the end of the recovery studies. Foliar concentration of Mg in seedlings kept for recovery studies increased from 0.7 to 1.9% at the end of the study period when the growth of seedlings was normal.

Discoloration of terminal leaves from dark green to pale green, which gradually advanced from margin inwards, was the typical symptom observed in S-deficient seedlings. Compared with the control, S-deficient seedlings had 25.7% less total chlorophyll content on the last month. Development of chlorosis, leaf curling and premature defoliation in teak seedlings due to S deficiency have also been reported by Kaul *et al.* (1972). Since, S is intimately associated with protein synthesis, its deficiency results in accumulation of carbohydrates and soluble N compounds, thereby resulting in a breakdown and decrease in cambial tissues. This might have resulted in the lanky appearance of seedlings (Lott *et al.* 1960). At the end of the study period, the collar diameter was found to be 39.5% lower compared with the control. Similarly the shoot fresh and dry weights due to S deficiency were respectively 47.0 and 36.6% lower when compared with the control. The S content in the foliage of these seedlings after the application of complete nutrient solution improved to 0.05%, when the visual symptoms started disappearing.

The initial symptoms of Zn deficiency appeared on the lower leaves as chlorotic patches. Seedlings developed short internodes, more number of branches and small, clustered leaves. Chlorophyll content also declined gradually in these seedlings as the study progressed. The leaf area of Zn-deficient seedlings was 66.7%

less compared with the control. The shoot fresh and dry weights also showed reduction by 46.3 and 36.3% respectively compared with the control. Typical interveinal chlorosis termed as mottled leaf reduced internodal length and little leaf are the common symptoms of Zn deficiency as reported by Greulach (1973). Zn deficiency results in inadequate supply of IAA, a very important growth hormone. In Zn-deficient seedlings, the concentration of Zn in the leaf tissues decreased gradually as visual symptoms progressed. The Zn content reduced from 0.013% in the beginning to 0.004% at the end of the study. The study also showed that N and K levels were high, while Ca levels decreased on account of Zn deficiency. Smith (1966) reported that Zn deficiency is often associated with high content of N and K and low content of Ca in citrus leaves. On the application of complete nutrient solution, the seedlings recovered well from the visual symptoms and growth retardation induced by Zn deficiency. The tissue level of Zn also improved significantly.

Symptoms of Mo deficiency appeared first on terminal leaves. Leaf size reduced considerably. The changes in isoenzyme pattern are the primary biochemical changes occurring at the cell level due to the deficiency of this element and on account of these physiological changes, the plant finally shows its effect on the leaf anatomical and morphological characters further expressing as visible deficiency symptoms (Kamala *et al.* 1988). Like other elements, Mo-deficient plants recovered well by the end of the study period when supplied with the complete solution. The growth was normal and there was improvement in discoloration and size of the leaves.

## Conclusions

Characteristic visual deficiency symptoms were manifested by the seedlings at different levels of deficiencies of N, P, K, Mg, S, Zn and Mo. Deficiency symptoms mainly include leaf discoloration, necrosis, scorching, defoliation and growth stunting. Reductions in height, collar diameter, leaf area, leaf number, shoot and root biomass were noticed for various levels of deficiencies of nutrient elements. The root growth and all the fractions of chlorophyll of treated seedlings particularly N-deficient seedlings declined considerably. Synergic and antagonistic effects of certain elements were also evident from the present study. Visual deficiency symptoms of seedlings coincided with marked reduction in foliar levels of the concerned elements. Foliar symptoms manifested by seedlings due to the deficiency of nutrients gradually disappeared during the recovery studies. Chemical analysis of leaf tissues at the end of the recovery studies revealed that the elemental concentrations of most of the nutrients in these seedlings were improved. The typical symptoms of deficiencies of various nutrient elements could be used as a guideline for diagnosing nutrient deficiencies of teak in commercial nurseries and plantations. The present study also showed the possibility of improving seedling growth and control of deficiencies of various nutrients by their proper application.

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