



RESEARCH PAPER

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Visual deficiency symptoms of Parashorea and Hopea species seedlings as affected by macronutrient omission

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Abstract

This study was conducted to assess and describe the visual nutrient deficiency symptoms exhibited by three dipterocarp seedlings as affected by mineral nutrient omission. A complete randomized design was used with 7 treatments and 4 replications. There were 36 seedlings per treatment per replication per species used in the experiment. The occurrences of deficiency symptoms in response to nutrient element omission on the study plants were monitored weekly. Photos on possible nutrient deficiency symptoms were taken typically on unusual colors or patterns in the leaves, burns, distortion of individual plant parts, stunting or abnormal growth. The photos were individually compared to several published literature for confirmation. Results showed that the common characteristics of macronutrient deficiency symptoms were chlorosis on the leaf blade, interveinal and marginal chlorosis, and scorching in the leaf tip. In the later stages, necrosis starts to develop after chlorosis. Furthermore, it was observed that deficiency symptoms depend on the plant species and the macronutrient content required for optimal growth. The absence of an essential macronutrient affects plant growth and performance. When the supply was suboptimal, the morphological growth performance of the six-dipterocarp seedlings grown under no fertilizer application and -N treatments were stunted. Therefore, nutrient element omission considerably influenced the growth performance of dipterocarp seedlings which showed nutrient deficiency symptoms specific to the omitted nutrient element.

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Introduction

The Dipterocarpaceae are the most important source of timber in Southeast Asia (ESSC, 1999). Hence, they are the ones used by the government's biggest environmental project, the National Greening Program (NGP). It plays a dominant role in the ecology and economics of the forests of Asia (Poore, 1989) in a way that no comparable family plays in other rainforest regions.

Plants need the right combination of nutrients to live, grow and reproduce. When plants suffer from malnutrition, they show symptoms of being unhealthy. Too little or too much of any one nutrient can cause problems (Hosier, 1999). To determine elemental plant deficiencies, most agriculturists rely primarily on visual symptoms, soil analysis, and plant tissue analysis (Wong, 2005). Visual nutrient deficiency symptoms have been described for a number of ornamentals (Joiner *et al.*, 1983) but limited study was conducted on trees, particularly Dipterocarpaceae.

Visual mineral deficiency symptoms vary and are often unique for different species (Harbaugh, 1986; Hershey and Merritt, 1987). Characterization of leaf development and nutrient deficiency symptoms could aid in diagnosing nutrient disorders and distinguishing nutrient imbalances from other disorders caused by pathogens, chemical damage, or other stresses. The objective of the study was to assess and describe the visual deficiency symptoms of N, P, K, Ca, Mg of the six-dipterocarp species as influenced by nutrient omission treatments under screen house conditions.

Materials and methods

Location and Climatic Condition of the Study Site

This study was conducted at the Terrestrial Ecosystems Division, Institute of Tropical Ecology and Environmental Management (ITEEM), Visayas State University, Visca, Baybay City, Leyte from June 2016 to May 2017. The average annual precipitation of the study area was 2620 mm and the mean annual temperature of 27.5°C.

The 1 pm measurement registered the highest temperature reading both inside (31.1 °C) and outside

(33.2 °C) and the difference in temperature between the inside and outside was about 1.7 °C. However, the relative humidity both outside and inside was generally the same and stable during the period of the experiment. Moreover, the average daily solar radiation of both the inside and outside location of the greenhouse is statistically different due to the net covering that was fitted right below the plastic roofing materials of the structure to reduce the availability of light inside the greenhouse. Hence, eliminating the effect of light will guarantee the extent of the specific-visual deficiency symptoms of the omitted nutrient on the study plants at the seedling stage.

Experimental Design and Layout of the Study

The experimental design of the study was Complete Randomized Design (CRD) with 7 treatments and 4 replications. There were 36 seedlings per treatment per replication per species used in the experiment which constituted a total of 252 seedlings per species. The seedlings were placed in an elevated wooden box (to prevent the roots from directly contacting the soil and were randomly distributed inside the greenhouse. The treatments were as follows: Treatment(T₁) 1 - without application of nutrient solution (control); Treatment(T₂) 2 - nitrogen (-N) is omitted in the solution; Treatment (T₃) 3 - phosphorus (-P) is omitted in the solution; Treatment (T₄) 4 - potassium (-K) is omitted in the solution; Treatment (T₅) 5 - calcium (-Ca) is omitted in the solution; Treatment (T₆) 6 - magnesium (-Mg) is omitted in the solution; and Treatment (T₇) 7 - complete (N, P, K, Ca and Mg) nutrient solution.

Preparation of Potting Media and Planting of Seedlings

The potting media used in planting the seedling was gravel. The gravels were washed with distilled water to remove the organic matter adhering to it and planted into a polyethylene bag with a dimension of 6"x10". A small amount of soil was added at the inside bottom of the polyethylene bag before adding the gravel to help the plant roots hold on to moisture. After 12 months, the seedlings were re-bagged in an 8"x12" dimension polyethylene bag. The planted seedlings were placed in a rectangular wooden box with a dimension of 1.3m x 0.7m to fit all the 36 seedlings.

Preparation of Nutrient Solution

The Hoagland's solution was used in the preparation of the nutrient solution. Each stock solution was prepared in separate containers. The molar mass of each chemical or salts in g/mol was calculated to attain the exact amount of the salt in grams needed in the preparation of 1 mol/L (1 M) stock solution. The exact amount of each chemical or salt was obtained using an analytical balance which was measured in grams. The salts were placed inside a 1000 mL volumetric flask, and then it was added with distilled water (half-filled). Furthermore, constant shaking was done to allow the salts to dissolve. Then, distilled water was filled again until the calibration line is reached. The preparation and the amount of the different salt composition for the micronutrient, complete, and the nutrient omission (-N, -P, -K, -Ca, and -Mg) stock solutions for a volume of 1 L were the following; Macronutrients: 101.11 KNO₃, 236.18 Ca(NO₃)₂·4H₂O, 246.37 MgSO₄·7H₂O, 132.97 NH₄H₂PO₄·H₂O. Micronutrients: 2.86H₃BO₃, 1.81MnCl₂·4H₂O, 0.22ZnSO₄·5H₂O, 0.02(NH₄)₂MoO₄·2H₂O), 0.08CuSO₄·5H₂O, 3.73Na₂EDTA, 1.78FeCl₂·4H₂O. Filler salts: 85.00NaNO₃, 147.02CaCl₂·2H₂O, 74.60KCl, 203.30MgCl₂·6H₂O, 142.10Na₂SO₄, 53.50NH₄Cl, 156.01NaH₂PO₄·H₂O.

Application of Nutrient Solution

A 20 ml complete nutrient solution was applied to the seedlings using a graduated syringe. This was applied on the surface of the potting medium to the study plants. To ensure equal distribution of the solution and to prevent salt injury, acetate with a circular shape with 8 holes on the side and 1 whole at the center was fabricated as a guide during the application of the nutrient solution.

Monitoring of Visual Deficiency Symptoms

The occurrences of visual deficiency symptoms in response to nutrient element omission on the study plants were monitored weekly. Likewise, photos of possible nutrient deficiency symptoms were taken. These are unusual colors or patterns in the leaves, burns, distortion of individual plant parts, stunting or abnormal growth. The photos were individually compared to several published literature (mostly on

agricultural crops or higher plants) for confirmation. After the validation, assessment and description of the visual deficiency symptoms were undertaken. On the other hand, plant growth parameters (morphology) were measured every 3 months; i.e., root-collar diameter and total height. The collar diameter was measured using a vernier caliper (mm) and total height was measured using a meter stick (cm).

Results and discussion

Seedling morphological growth performance

Marschner (1995) reported that depending on the plant species, development stage, and organ, the macronutrients content required for optimal growth varied. The absence or too much of essential plant nutrients affects plant growth and performance. When the supply is suboptimal, growth is retarded. Generally, the growth performance of six-dipterocarp seedlings was uniform during the first two months after treatment application. Variations in the general stand of plants became noticeable starting the months after treatment application when some of the nutrient-deficient plants such as -N showed symptoms of deficiency.

Parashorea malaanonan

As can be seen in Appendix Fig. 1A, *Parashorea malaanonan* seedlings grown under control (no fertilizer application) and -Ca treatments have the shortest height among the seven treatments after 12 months of treatment application.

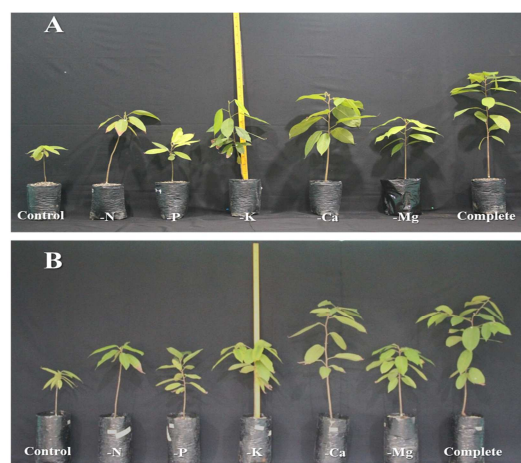


Fig. 1. Morphological growth performance of *Parashorea malaanonan* seedlings as affected by different nutrient element omission after 12 (A) and 18 (B) months of treatment application.

According to Uchida (2000), calcium usually limits growth. However, after 18 months (Fig. 1B) of treatment application, *Parashorea malaanonan* seedlings planted under -K and complete fertilizer application treatments did not show any substantial difference in plant height and leaf area (Yeh *et al.*, 2000).

Hopea plagata

As can be seen in Appendix Fig. 2A, *Hopea plagata* seedlings grown under control (no fertilizer application) and -N treatments have the shortest height among the seven treatments after 12 months of treatment application. According to Roy *et al.* (2006) nitrogen deficiency most often results in stunted growth, slow growth, and chlorosis. However, after 18 months (Fig. 3B) of treatment application, -K, -Mg, and completion did not show any substantial difference in plant height.

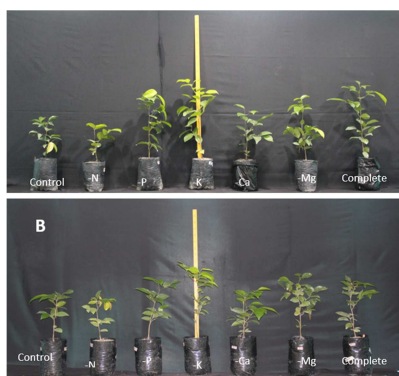


Fig. 2. Morphological growth performance of *Hopia plagata* seedlings as affected by different nutrient element omission after 12 (A) and 18 (B) months of treatment application.

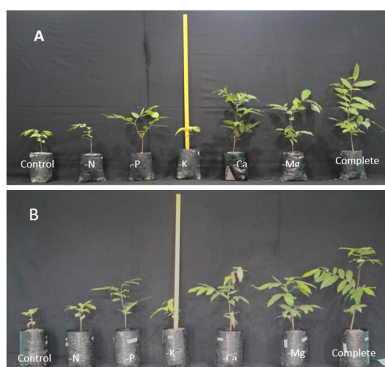


Fig. 3. Morphological growth performance of *Hopia philippinensis* seedlings as affected by different nutrient element omission after 12 (A) and 18 (B) months of treatment application.

Hopea philippinensis

As shown in Appendix Fig. 4A, after 12 months of treatment application, the control (no fertilizer application) and -K depicts stunted growth, while complete (with N, P, K, Ca and Mg application) treatment obtained the highest height growth among *Hopea philippinensis* seedlings. Due to the availability of macronutrient present in the medium of complete fertilizer treatments, it had resulted in longer plant height. A similar trend was observed in the study plants after 18 months of treatment application, (Fig. 4B). Patterns of yellow to brown colors and spots and necrosis on the veins and leaf blade were observed. This was also true in the study of Yeh *et al.* (2000) shows that due to nitrogen deficiency, the leaf initiation, leaf area, and matured leaf sizes have reduced. As a result, in N deficiency, from pale yellow leaves on older leaves to light yellow leaves and in later stages necrotic areas which are usually exhibited in the veins are observed (Hodges and Constable, 2010; Grusak *et al.*, 2001).

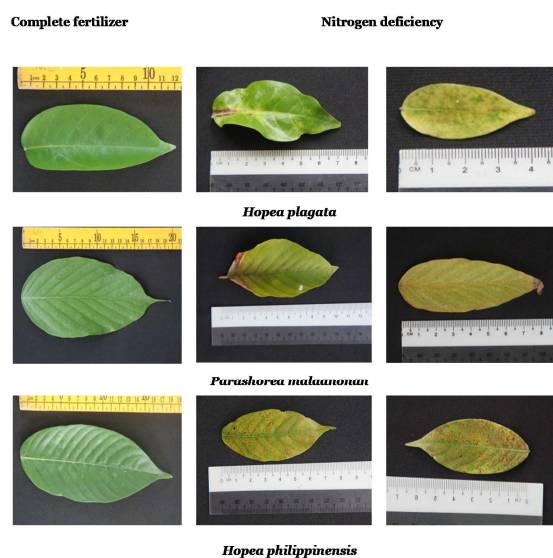


Fig. 4. Visual deficiency symptoms of nitrogen in the leaves of the six-dipterocarp species at seedling stage.

Unusual Colors or Patterns in the Leaves

Nutrients absorbed by the plants accumulated in the leaves regardless of whether the nutrients were supplied in the medium or not. Marschner (1995) reported that when the supply is suboptimal, growth is retarded; nutrients are mobilized in mature leaves and retranslocated to areas of new growth.

Nitrogen

Symptoms of N deficiency became evident as early as three months after planting. Appendix Fig. 4 presents the deficiency symptoms of nitrogen observed on the leaves of the six-dipterocarp species at the seedling stage. The common characteristics of N deficiency symptoms documented were yellowing of the leaves including the veins of the six study plants. Patterns of yellow to brown colors and spots and necrosis on the veins and leaf blade were observed.

The deficiency symptoms observed in this study were similar on the results of the study conducted by Yeh et al. (2000) where due to nitrogen deficiency, the leaf initiation, leaf area, and matured leaf sizes have reduced. As a result, in N deficiency, from pale yellow leaves on older leaves to light yellow leaves and in later stages necrotic areas which are usually exhibited in the veins are observed (Hodges and Constable, 2010; Grusak et al., 2001). Lastly, enhanced senescence was observed in older leaves (Marschner, 1995) due to the decline in net photosynthesis (Hodges and Constable, 2010).

Phosphorus

Phosphorus (P) is an essential macronutrient that constitutes about 0.2 % of the plant's dry matter (Marschner, 1995). In plants, Phosphorus (P) is considered second to nitrogen as the most essential nutrient to ensure health and function. Phosphorus is used by plants in numerous processes such as photophosphorylation, genetic transfer, the transportation of nutrients, and phospholipid cell membranes. When phosphorus is present at inadequate levels, genetic processes such as cell division and plant growth are impaired. Hence, phosphorus-deficient plants may mature at a slower rate than plants with adequate amounts of phosphorus.

Appendix Fig. 5, presents the common characteristics of P deficiency symptoms in the leaves of the six-dipterocarp species at the seedling stage. These symptoms were; darker green leaves and purplish or red pigment. The older leaves were affected first and may acquire a purplish discoloration due to the accumulation of sugars which favors anthocyanin

synthesis; in some cases, leaf tips will brown and die (McCauley, 2011).

Plants suffering from P deficiency appear weak and maturity is delayed. Leaf expansion and leaf surface area may also be inhibited, causing leaves to curl and be small (McCauley, 2011). These symptoms were also observed in all of the study plants specifically those grown under -P treatment.

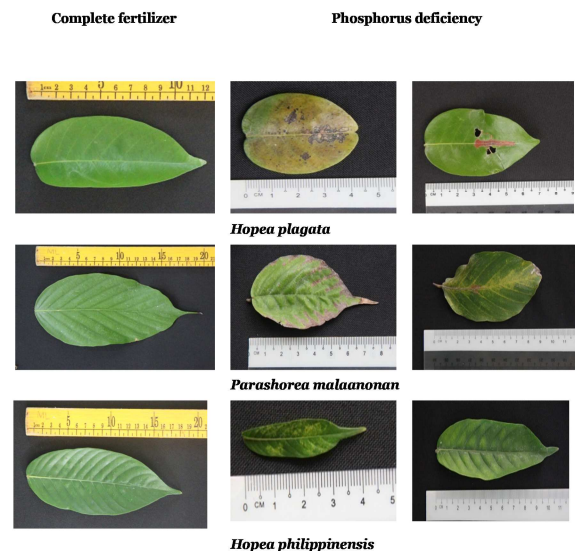


Fig. 5. Common characteristics of Phosphorus deficiency symptoms in the leaves of the six-dipterocarp species at seedling stage.

Potassium

Potassium is utilized by plants in the activation of enzymes, photosynthesis, protein formation, and sugar transport. Initially, there is only a reduction in growth rate, with chlorosis and necrosis occurring in later stages (Mengel and Kirkby, 2001). Affected older leaves will show localized mottled or chlorotic areas with leaf burn at the margins. Chlorotic symptoms typically begin on the leaf tip, but unlike the 'V' effect caused by N deficiency, K deficient chlorosis will advance along the leaf margins towards the base, usually leaving the midrib alive and green (Fig. 6).

As the deficiency progresses, the entire leaf will become yellow (McCauley, 2011). Small white or yellow necrotic spots also developed, beginning along leaf margins. These were documented in *Hopea plagata* and *Parashorea malaanonan* seedlings.

Moreover, all of the study plants exhibited yellow or purple leaf tints with browning at the leaf edge. Dead areas near the tips and margins of leaves were observed among the study plants.

Meanwhile, yellowing at the leaf margins which continued towards the center was observed in *Parashorea malaanonan* and *Hopea philippinensis*. Scorching appears to have exhibited at the tip of the leaves (Hodges, 2010) and leaves appear to have marginal scorching (Pallardy, 2010). This symptom occurs first in the older leaves because K is very mobile in the plant.

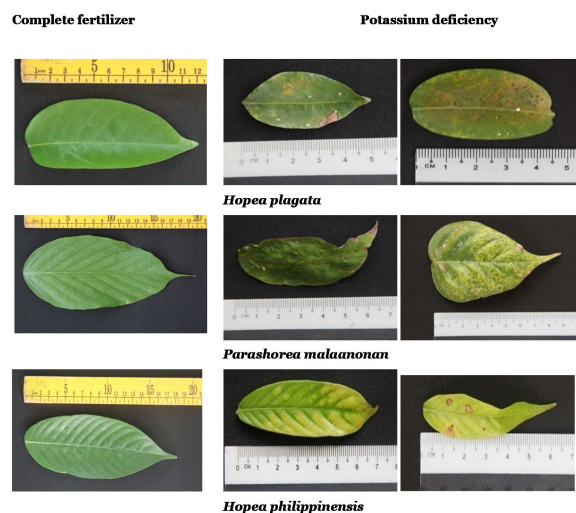


Fig. 6. Common characteristics of Potassium deficiency symptoms in the leaves of the six-dipterocarp species at seedling stage.

Calcium

Calcium is a component of plant cell walls and regulates cell wall construction (McCauley, 2011). Insufficient Ca can cause young leaves to become distorted and turn abnormally dark green (McCauley, 2011). This was observed in the leaf of *Hopea plagata* (Fig. 7). According to Yeh *et al.* (2000), the dark green appearance of the leaves was due to the increased chlorophyll content.

Moreover, most of the leaf tips of the sample plants were often hooked-shape, dry or brittle, and eventually wither and die. O'Sullivan *et al.* (1997) stated that the primary symptom of Ca deficiency is the development of necrotic tissue on the leaves. He

described that the necrosis usually begins along the lateral margins, and extends inward mainly in interveinal tissue is not usually preceded by localized chlorosis, although the leaves may be uniformly paler than normal. This was observed in all of the study plants in both 12 and 18 months after treatment application. Furthermore, in this study, necrosis was first noted on young expanding leaves, two or three leaves below the tip, but as the disorder intensifies, newly formed leaves were affected, and finally, the apex dies. Finally, scorching in the marginal leaf or in the tip of the leaves and deformities in the shape of the leaves were observed in the Ca-deficient plant treatment. Spots from orange to brown coloration were found on the leaf which was probably the result of desiccation of the cells in the leaf (Hodges and Constable, 2010; Marschner, 1995; Hepler, 2005).

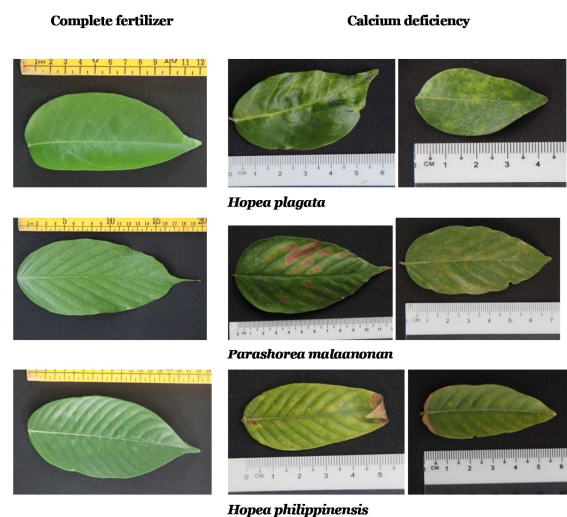


Fig. 7. Common characteristics of Calcium deficiency symptoms in the leaves of the six-dipterocarp species at the seedling stage.

Magnesium

Magnesium is the central molecule in chlorophyll and is an important co-factor for the production of ATP. McCauley (2011) reported that symptoms of Mg deficiency include interveinal chlorosis and leaf margins becoming yellow or reddish-purple while the midrib remains green.

As can be seen in Fig. 8, the leaves of *Parashorea malaanonan* and *Hopea philippinensis* were

yellowish between the veins while the midrib remains green. Likewise, the curling of the leaves and red patches of *Shorea almon* seedlings was the clear symptom of Mg deficiency in this study. This symptom was documented in wheat and alfalfa (McCauley, 2011).

On the other hand, due to magnesium's mobile nature, the plant will first break down chlorophyll in older leaves and transport the Mg to younger leaves which have greater photosynthetic needs. After prolonged magnesium deficiency, necrosis and dropping of older leaves occur. Similar observations were documented in this study in all of the six-dipterocarp seedlings particularly those planted under -Mg treatment.

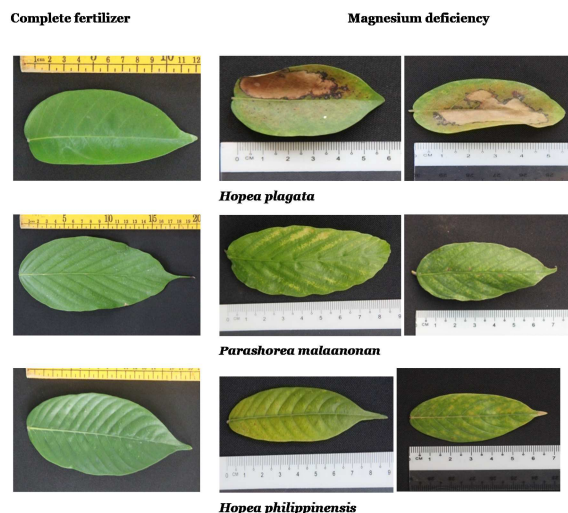


Fig. 8. Common characteristics of Magnesium deficiency symptoms in the leaves of the six-dipterocarp species at seedling stage.

Conclusions

This study was conducted to assess and describe the visual deficiency symptoms of the three dipterocarp seedlings as affected by macronutrient omission under greenhouse conditions. Based on the results of this study, the following conclusions are generated:

- The common characteristics of nitrogen deficiency symptoms observed were the appearance of uniform yellowing of the leaves including the veins and stunted growth among the six study plants;
- Phosphorus-deficient plants showed darker green leaves and purplish or red pigment while older leaves

exhibited purplish discoloration and localized mottled or chlorotic areas with leaf burn at margins;

c) Potassium-deficient seedlings exhibited chlorosis along the leaf margins towards the base, usually leaving the midrib alive and green;

d) Calcium deficiency symptoms observed where young leaves became distorted and leaf tips were often hooked-shape, dry or brittle and eventually die;

e) Magnesium deficiency symptom includes interveinal chlorosis and the leaf margins becoming yellow or reddish-purple while the midrib remains green;

Therefore, nutrient element omission considerably influenced the growth performance of the six-dipterocarp seedlings which exhibited nutrient deficiency symptoms specific to the omitted nutrient element.

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