









ORIGINAL ARTICLE

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## Quality of Indian cedar seedlings grown under micronutrient omission

### *Qualidade de mudas de cedro indiano cultivadas por omissão de micronutrientes*

**ABSTRACT:** The need to improve silvicultural techniques, which can contribute to increasing forest productivity, arises due to the growing demand for products derived from wood. The mineral fertilization is highlighted as essential among factors that influence the initial growth of plants, the reason why the knowledge on the nutritional requirements of forest species is found to be important. The objective of this work was to evaluate the effect of micronutrient omission on biometric parameters in seedlings of *Acrocarpus fraxinifolius* Wight & Arn., (Indian cedar) grown in nutrient solution. The study was established in a completely randomized design, with seven treatments, four replications and one plant per pot. Treatments consisted of the complete solution of nutrients Hoagland and Arnon and omission of the following selected micronutrients: boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn). At 90 days after transplantation, the following biometric parameters were evaluated: height of the shoot, diameter of the stem, dry mass of the shoot and root. The visual symptoms of micronutrient deficiency were also evaluated, as well as the content and accumulation of nutrients in the aerial part of the seedlings. The deficiency of micronutrients in the nutrient solution, showed a negative effect on the growth of seedlings of *Acrocarpus fraxinifolius* Wight & Arn. The limiting micronutrients in the growth of Indian cedar seedlings in decreasing order were Fe>Cu>B>Mn>Mo>Zn.

**RESUMO:** A necessidade de aprimoramento das técnicas silviculturais, que podem contribuir para o aumento da produtividade florestal, surge devido a demanda crescente de produtos derivados de madeira. A fertilização mineral é destacada como essencial entre os fatores que influenciam o crescimento inicial das plantas, razão pela qual o conhecimento sobre as exigências nutricionais das espécies florestais se mostra importante. O objetivo deste trabalho foi avaliar o efeito da omissão de micronutrientes sobre parâmetros biométricos em mudas de *Acrocarpus fraxinifolius* Wight & Arn., (cedro indiano) cultivadas em solução nutritiva. O estudo foi estabelecido em delineamento inteiramente casualizado, com sete tratamentos, quatro repetições e uma planta por vaso. Os tratamentos consistiram na solução completa de nutrientes Hoagland e Arnon e na omissão dos seguintes micronutrientes selecionados: boro (B), cobre (Cu), ferro (Fe), manganês (Mn), molibdênio (Mo) e zinco (Zn). Aos 90 dias após o transplante foram avaliados os seguintes parâmetros biométricos: altura da parte aérea, diâmetro do coleto, massa seca da parte aérea e radicular. Também foi avaliada a sintomatologia visual da deficiência de micronutrientes, bem como o conteúdo e acúmulo de nutrientes na parte aérea das mudas. A deficiência dos micronutrientes na solução nutritiva influenciou negativamente o crescimento das mudas de *Acrocarpus fraxinifolius* Wight & Arn. Os micronutrientes limitantes do crescimento das mudas de cedro indiano em ordem decrescente foram Fe>Cu>B>Mn>Mo>Zn.

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## 1 Introduction

The demand for wood-based forest products has led to a constant search for new silvicultural techniques, with the aim of increasing the productivity of forest plantations. Aspects such as genetic improvement, adaptation to the climate, appropriate management practices, in addition to the insertion of new technologies in the forestry area, are considered essential for gains in the increase of forest stands. Among these techniques, the use of fertilizers stands out as one of the main means employed to provide increased productivity in the forest area (Momolli et al., 2019).

Researches have been carried out using different doses of fertilizers, aiming to increase plants growth and the productivity of forest species (Venturin et al., 2014; Sousa et al., 2018). *Eucalyptus* genera are highlighted among the most studied forest species, which have been planted in several regions of Brazil, mainly due to their rapid growth and satisfactory productivity, and also because they are the basis of several products that supply the market in the forestry area (IBÁ, 2019).

Studies have recently been declining for new species such as *Acrocarpus fraxinifolius* Wight & Arn., (Indian cedar), which has aroused the interest of Brazilian researchers for also presenting rapid growth, due to the diversification of their use in the timber industry. Besides, this forest specie has been implemented in the formation of agroforestry systems, as well as in the recovery of degraded areas (Gonçalves et al., 2012; Venturin et al., 2014; Firmino et al., 2015).

*Acrocarpus fraxinifolius* Wight & Arn., is found naturally in mixed evergreen forests of India, Bangladesh, Indonesia, Nepal and Myanmar, between latitudes 23° and 27° North, distributed from sea level up to 1,500 m altitude. Its distribution occurs in areas with temperatures between 19° and 28°C and annual precipitation ranging from 1,000 to 2,000 mm. This species shows better silvicultural performance in deep, clayey, and well-drained soils, with pH from 5 to 7 (Venturin et al., 2014; Silva et al., 2016).

Indian cedar is a large tree of the Fabaceae family, which has been used as a shade tree in coffee plantations, as well as for wood production and / or forest enrichment. It produces hard wood, but easy to work with, which is used, for instance, in the manufacture of fine furniture, application in buildings for interior finishing, manufacture of musical instruments and boxes for packaging (Firmino et al., 2015).

The current state of knowledge of the silvicultural behavior of *Acrocarpus fraxinifolius* Wight & Arn., is focused on silvicultural aspects, management of plantations and the use of the specie. However, there is not enough information on nutritional requirements in the initial growth phase of this species in Brazil (Mishra et al., 2015). Thus, considering that fertilization practices are fundamental for the production process of high quality seedlings and to enable future high-productivity forest plantations (Gonçalves et al., 2012; Mishra et al.,

2015), carrying out studies on fertilization of potentially producing species such as *Acrocarpus fraxinifolius* Wight & Arn., is found to be essential.

The study of rationalization of nutritional demands of plants in the production process of cedar seedlings may effectively contribute to the increase in knowledge about interactions of nutrients on plant growth and the later use of these seedlings to form productive forests. In this regard, one of the ways used to assess the influence of nutrients on initial growth is the technique of the missing element.

This technique is used to assess nutritional requirements of plant species, determining soil nutritional deficiencies in real field or greenhouse conditions, based on the law of the minimum (Browne, 1942), which consists of testing a complete treatment of nutrients, together with treatments based on individual omissions of other nutrients (Malavolta, 1980).

Although signal transduction pathways and plant sensing for mineral deficiencies are more known for macronutrients, the knowledge about micronutrients is still limited. The use of selected micronutrients can contribute to the initial growth of seedlings. In this context, this work aimed at evaluating the effect of omission of micronutrients on biometric parameters in seedlings of *Acrocarpus fraxinifolius* Wight & Arn., grown in nutrient solution.

## 2 Material and Methods

The experiment was carried out in a greenhouse at the Viveiro Florestal of the Federal University of Lavras (UFLA) located in Lavras, State of Minas Gerais, Brazil, at 21°13' 14, 033" S, 44° 58' 0,232" O. Seeds of *Acrocarpus fraxinifolius* Wight & Arn., used for seedlings production were collected from four parent plants selected at the UFLA Historical Campus, distant at 100 meters from each other. The best seeds were then selected and packed in plastic trays for further cleaning and scarification. Seeds were sown in tubes of 55 cm<sup>3</sup> containing vermiculite substrate and moistened with deionized water.

After reaching approximately 10 cm height, about 30 days after sowing, seedlings were washed with deionized water in bare roots and transplanted to a plastic tray containing 20 liters of complete nutrient solution Hoagland & Arnon (1950). The nutrient solution was kept under constant aeration with compressed air to maintain the air flow and oxygenate the hydroponic nutrient solution.

This solution was at 30% of its ionic force and seedlings were kept for 15 days in each solution. Then, the nutrients solution was changed and adjusted to 60% of its ionic force. After these adaptation days, each seedling was transplanted in a 5 liters pot containing nutritive solution at 100% of its ionic force, and put on a stand under constant aeration. The pH was about 5.5 and seedlings were then fixed by means of the stem with the help of polystyrene sheets about 2 cm thick.

The experiment was established in completely randomized design with seven treatments, four replications, and one plant per pot. Treatments consisted of complete nutrient solution Hoagland and Arnon, and omissions of selected single micronutrients, namely boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn), based on the law of minimum (Browne, 1942).

Analytical reagent and deionized water were used to prepare nutrient stock solutions. Plants were daily monitored and the solution volume was completed using deionized water whenever it was necessary, and the nutrient solution was changed biweekly. Seedlings were constantly monitored to diagnose nutrient deficiency symptoms under test, and the common symptoms of each micronutrient were recorded at seedlings harvest.

At 90 days after the transplant, shoot height (H) and root collar diameter (CD) were measured. Then, plants were harvested, separated into shoots and roots, and washed in running water and deionized water. After, plants were dried in a forced air heating system (hothouse) at 65°C temperature for three days. The plant material was then weighted on precision scale to estimate the following biometric parameters: shoot dry mass (SDM), root dry mass (RDM), and total dry matter (TDM). The ratio given by RDM and SDM was obtained, as well as the Dickson quality index (DQI) given by the equation (1) according to Dickson et al. (1960).

$$DQI = \frac{TDM(g)}{H(cm)/CD(mm) + SDM(g)/RDM(g)} \quad (1)$$

Where: TDM is the total dry mass; H is the height; CD is the diameter of the collection; SDM is the dry mass of the aerial part; and RDM is the dry mass of the root.

The dry matter of the aerial part was ground in a laboratory mill of the Wiley type, Logen Scientific brand - model WLS-3004, and submitted to sulfuric and nitric-perchloric digestion to estimate the micronutrient content, as described in Malavolta et al. (1997). The Azomethine H colorimetric method was used to determine the B dissolved in the water, in which the B is complexed with azomethine H. The content of Cu, Fe, Mn and Zn was estimated from nitric-perchloric extracts by means of the analytical technique using the atomic absorption spectrophotometer, Shimadzu, model AA-7000, which analyzes the concentration of elements in a liquid sample based on the absorbed energy of certain wavelengths of light.

The Sisvar version 5.6 (Ferreira, 2014), a computer statistical analysis system, was used for data analysis. The analysis of normality of errors was carried out by the Shapiro-Wilk test at 5% significance level, and once all assumptions were made, the analysis of variance (ANOVA) was carried out to visualize whether there exists any significant difference between multiple sample

means. Then, means were compared by the Scott and Knott test at 5% significance level.

### 3 Results and Discussion

Statistically significant differences were found between treatments (Table 1). Treatments consisting of the omission of isolated micronutrients B, Cu and Fe were the most limiting factors for the growth of seedlings of *Acrocarpus fraxinifolius* Wight & Arn. Results described by Wallau et al. (2008), Sorreano et al. (2008) and Silva et al. (2016) point out that the deficiency of Fe, Cu and B, influences the growth of different plant species.

In this work, the omission of Mn, Mo and Zn, did not cause significant effect on biometric parameters under study, when compared to the complete treatment. Such fact may have occurred because of the low demand of these micronutrients by cedar seedlings, demonstrating that these micronutrients cannot be considered limiting factors in the first growing stages of seedlings of indian cedar (Dias et al., 2020).

According to Marschner (2012) and Lima et al. (2017) the deprivation of a single selected micronutrient results in a decrease in its concentration in plant tissues, and the acute deficiency of this micronutrient can trigger symptoms in the leaves, with a negative effect on the growth rate of the seedlings. However, micronutrients have a different influence on seedling growth, which also depends on their concentration in the solution used (Silva et al., 2016; Dias et al., 2020).

**Table 1.** Mean values of biometric parameters obtained for *Acrocarpus fraxinifolius* Wight & Arn., seedlings, grown under micronutrients omission 90 days after the transplant.

**Tabela 1.** Valores médios dos parâmetros biométricos obtidos para mudas de *Acrocarpus fraxinifolius* Wight & Arn., cultivadas sob omissão de micronutrientes 90 dias após o transplantio.

Treatment	H (cm)	CD (cm)	SDM (g)	RDM (g)	TDM (g)	SDR/RDM -	DQI -
Complete	87.87 a	16.07 a	78.27 b	12.18 a	90.45 b	6.59 a	7.56 a
- B	51.87 b	10.97 c	21.78 c	7.04 b	28.83 c	3.22 b	3.63 c
- Cu	52.37 b	13.55 b	32.94 c	9.05 b	41.99 c	3.65 b	5.59 b
- Fe	10.75 c	2.95 d	1.27 d	0.32 c	1.59 d	4.58 b	0.20 d
- Mn	96.62 a	17.20 a	80.37 b	13.57 a	94.45 b	6.16 a	8.06 a
- Mo	103.70 a	17.50 a	95.26 a	14.48 a	109.70 a	6.57 a	8.75 a
- Zn	97.62 a	18.77 a	91.61 a	16.08 a	107.60 a	5.80 a	9.94 a
SD	34.30	5.49	37.82	5.42	43.07	1.40	3.38
CV (%)	12.71	10.77	18.22	21.54	17.01	26.25	19.40

Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. H = shoot height, CD = root collar diameter, SDM = shoot dry mass, RDM = root dry mass, TDM = total dry matter, and DQI = Dickson quality index. SD = Standard deviation e CV = Coefficient of variation.



The treatment consisting of omission of B showed a significant effect on cedar seedlings growth, for all evaluated parameters (Table 1). Similar results were described by Sorreano et al. (2008), when evaluating the micronutrient deficiency in seedlings of *Croton urucurana*. In addition, Anjos et al. (2018), assessing the growth of seedlings of *Cassia grandis*, found that the supply of B is one of the factors that enable greater root development in the initial plant growth stage, the reason why its use is also found to be important for better growth of seedlings in initial phase.

Cu deficiency caused a significant restrictive effect on the growth of cedar seedlings, mainly for the height of the seedlings (40.4%), dry mass of the aerial part (57.9%), total dry mass (53.6%) and for the RDM / SDM ratio (44.6%). Similar patterns were observed in other studies for the species *Croton urucurana*, *Khaya ivorensis* and *Dipteryx alata*, respectively (Sorreano et al., 2008; Wallau et al., 2008; Silva et al., 2016).

The deficiency of Cu can cause disturbances in plant growth and development, adversely affecting important physiological processes (Wallau et al., 2008; Silva et al., 2016). In this study, it was observed that the deficiency of this micronutrient caused a change in the architecture of the root and leaves. Typical copper deficiency symptoms first appeared at the tips of young leaves and then extended downwards along the leaf margins (Marschner, 2012; Coelho et al., 2020).

Treatments consisting of Fe omission were the most limiting factors for plant growth in this study, negatively influencing most of the evaluated parameters. The deficiency of this micronutrient caused chlorosis in new leaves, causing death in most of the seedlings, about 25 days after the transplant, probably due to disturbances in breathing, photosynthesis and other metabolic processes. According to Silva et al. (2016), Fe is one of the most limiting micronutrients for plant growth and metabolism, mainly due to the low solubility of the oxidized form of iron in aerobic environments.

Regarding micronutrient deficiency symptoms in the Indian cedar seedlings, grown in nutrient solution, seedlings showed visual deficiency symptoms at different times. The first common symptoms were observed for treatments with Fe and B omission at 15 days after transplantation, followed by treatments with Mn, Mo, Cu and Zn omission, 60 days after transplantation (Figure 1).

Seedlings grown under deficiency of B showed smaller plants with small leaves and leaflets (Figure 1A), and death of shoots and tips of leaves, resulting in the loss of apical dominance of seedlings. This might be because boron deficiency symptoms arise from disturbance of the cell wall structure, which caused disturbance in the cell elongation as described in Marschner (2012). B deficiency affects vegetative growth of plants resulting in inhibition of cell expansion, death of meristem and reduced growth (Sorreano et al., 2008; Sousa et al., 2018).

Seedlings cultivated in nutritive solution with absence of copper showed small leaves and leaflets and less consistency of petiole, with the plants also showing faded aspect (Figure 1B). Cu participates in several

physiological processes and is an essential cofactor for many metalloproteins (Malavolta et al., 1997). However, copper is immobile, which means that its deficiency symptoms occur in the youngest leaves, although they vary depending on the crop.



**Figure 1.** Symptomatology of micronutrient deficiency in *Acrocarpus fraxinifolius* Wight & Arn., seedlings grown in nutrient solution. The left side of each picture shows plants grown in complete nutrient solution, and the right side shows solution formulated with deficiency of boron (A), copper (B), iron (C) manganese (D), molybdenum (E), and zinc (F).

**Figura 1.** Sintomatologia da deficiência de micronutrientes em mudas de *Acrocarpus fraxinifolius* Wight & Arn., cultivadas em solução nutritiva. O lado esquerdo de cada figura mostra plantas cultivadas em solução completa de nutrientes, e o lado direito mostra a solução formulada com deficiência de boron (A), cobre (B), ferro (C) manganês (D), molibdênio (E) e zinco (F).

Usually, symptoms of Cu deficiency start with a mild chlorosis of the whole leaf or between the ribs of the young leaves. Within the chlorotic areas of the leaf, small necrotic spots may form, especially on the leaf margins (Hoffmam et al., 2019; Coelho et al. 2020). In this study, as the symptoms progressed, the youngest leaves decreased in size, lost their shine and, in some cases, the leaves withered. The apical meristems of the seedlings became necrotic and died, inhibiting the growth of the lateral branches, consequently affecting the growth of seedlings.

Fe also had high influence on seedling growth. Its deficiency was considered one of the most limiting factors in the growth of cedar seedlings. Plants showed yellow leaves, of low growth, causing death of seedlings 25 days after transplantation (Figure 1C). Similar data were observed by Hoffmam et al. (2019) with the species *Copaifera langsdorffii*, in its early growth phase. Malavolta et al. (1997) and Sorreano et al. (2008) stated that the most observed symptom of iron deficiency in plants is commonly called leaf chlorosis, where leaves turn yellow but the leaf ribs remain green. In this study, leaf chlorosis started at the tips and advanced to the older leaves of the seedling as the deficiency worsened.

Fe is essential micronutrient for the production of chlorophyll, so its deficiency can cause chlorosis in the leaves of the seedlings (Sorreano et al., 2008). This occurs mainly because iron is not mobile in the plant. Young leaves cannot extract iron from older leaves. With a serious shortage of this micronutrient, the older leaves and the smaller ribs of the leaf may also turn to yellow,

impairing the death of the seedlings (Wallau et al., 2008; Taiz & Zeiger, 2013).

Seedlings grown under omission of Zn showed some variable pattern of chlorosis on the young leaves (Figure 1F) and necrotic stains on the margins or tips of the leaves. Young leaves were smaller in size and often shell-shaped or distorted. These symptoms occurred because zinc is used in the formation of chlorophyll and some carbohydrates, conversion of starches into sugars (Neves et al., 2004; Wallau et al., 2008), as well as in the formation of auxins, which help in the regulation of growth and elongation of stems. Neves et al (2004) and Araújo et al. (2017) state that zinc has low mobility, and deficiency symptoms occur in new leaves and vary according to culture, so the correct diagnosis of deficiency symptoms is very important.

Concentrations of essential elements in plants may exceed critical concentrations, the minimum concentrations necessary for growth and may vary somewhat from species to species (Marschner, 2012; Dias et al., 2020). When a nutrient is missing or deficient, this deficiency causes anomalies due to changes in the plant's metabolism. Thus, the missing element technique provides semiquantitative micronutrient-related data that can limit plant development (Silva et al., 2016).

Micronutrients are required only in very small amounts and their concentrations in plant tissues are only a small proportion of the macronutrient concentrations (Malavolta et al., 1997; Martins et al., 2020). As most of the micronutrients are relatively immobile in the plant, they are not easily transferred from the older leaves to the younger ones. Therefore, the concentration of the nutrient tends to be lower on the younger leaves. Symptoms of deficiency are more pronounced on the young leaves that develop after the decrease in nutrients supply (Coelho et al., 2020), as occurred with the deprivation of selected micronutrients in a nutrient solution described in this study.

In the evaluation of the levels of micronutrients accumulated in the leaves of the seedlings, it was found that the levels of B, Cu, Mn and Zn increased in the treatment consisting of the omission of Fe, when compared to the complete nutrient solution (Table 2). These may have been caused by the effect of concentration (Corcioli et al., 2016; Silva et al., 2016), which may partially explain the inhibition of uptake of a certain ion, with effect on low biomass production in Indian cedar seedlings.

The B content in the aerial part of *Acrocarpus fraxinifolius* Wight & Arn., seedlings increased significantly in the treatment consisting of Mn omission. This effect may be due to the following situations, respectively: (i) lower biomass accumulation, which increased B concentration in plant tissues; and (ii) dilution effect from which, with the deprivation of Mn, a relative dry matter accumulation rate decreased faster than the B accumulation rate, resulting in higher final concentrations.

In other hand, the Cu content decreased significantly in treatments consisting of omission of Zn, as a result of

the concentration effect, as observed by Silva et al. (2016). This effect means that when seedlings were exposed to Zn deficiency, the total dry matter accumulation also decreased, causing imbalance in seedling growth (Araújo et al., 2017).

**Table 2.** Content of micronutrients of shoots of *Acrocarpus fraxinifolius* Wight & Arn., seedlings grown in nutritive solution under micronutrients omission.

**Tabela 2.** Teor de micronutrientes em mudas de *Acrocarpus fraxinifolius* Wight & Arn., cultivadas em solução nutritiva por omissão de micronutrientes.

Treatment	Content of micronutrients (mg kg <sup>-1</sup> )				
	Boron	Copper	Iron	Manganese	Zinc
Complete	83.52 b	22.83 b	24.87 a	85.95 b	93.70 b
- B	60.97 c	13.09 c	44.92 a	43.27 b	132.15 b
- Cu	80.75 b	10.92 c	46.00 a	68.95 b	73.12 b
- Fe	94.46 a	36.10 a	15.80 a	900.85 a	265.48 a
- Mn	93.76 a	8.03 c	56.77 a	18.92 b	33.05 b
- Mo	91.01 a	4.74 d	65.32 a	30.02 b	24.22 b
- Zn	82.52 b	2.90 d	56.12 a	41.55 b	3.55 b
SD	11.51	11.69	18.15	23.10	22.56
CV (%)	9.13	21.70	18.89	24.07	22.33

Means followed by the same letter in the column do not differ statistically from each other by the Scott-Knott test at 5% probability. SD = Standard deviation e CV = Coefficient of variation.

In general, the influence of micronutrients varied among the formulated treatments, with seedlings showing different growth rates and quality standards. Therefore, the use of micronutrients for fertilization of seedlings in the initial phase of growth should only be recommended after the laboratory testing of the substrate to prove its requirement for plants growth. In this regard, checking which micronutrients and levels should be formulated with macronutrients for plants fertilization is important for better seedlings growth and higher survival rates.

## 4 Conclusion

The growth of *Acrocarpus fraxinifolius* Wight & Arn., seedlings was influenced by the omission of micronutrients in the solution.

The limiting micronutrients for the growth of Indian cedar seedlings in decreasing order were Fe>B>Cu>Mn>Mo>Zn.

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