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Growth and visual symptoms of nutrient deficiencies in young *Mentha piperita* plants

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Abstract

The characterization of the deficiency symptoms of macronutrients and micronutrients on the growth of young plants of *Mentha piperita* were evaluated by the missing element technique. The experiment was carried out under greenhouse conditions at Universidade Federal Rural da Amazônia. The experimental design was completely randomized blocks with 12 treatments and four replicates and a total of 48 plots. The following treatments were tested: (C) Complete (macro-and micro-nutrients) and omissions of N, P, K, Ca, Mg, S, B ,Cu, Fe, Mn and Zn. The variables analyzed were: height, stem diameter, leaf area, and dry matter production. The omissions of nutrients induced morphological changes translated into deficiency symptoms. The omission of N and Ca showed the first visual symptoms of deficiency, followed by P, K, Mg, S, B, Cu, Fe and Mn=Zn; omissions of N, P, K, Mg, S, B, Cu, Fe, Zn and Mn = compromise the plant growth. The most limiting nutrients for the plant growth based on the total dry matter and foliar area was N, followed by Ca; boron is the micronutrient that most affects the plant growth.

Key words: *Mentha piperita*, mineral nutrition, nutritional deficiency.

Introduction

Mentha piperita L., a native plant to tropical regions, belongs to the family Lamiaceae, perfectly adapted to the climatic conditions of Amazon region. This plant has been used for decades by industries in the manufacture of perfumes, essences and cosmetics. It is common tourists visit the state of Para in search for those fragrances to introduce it in other countries, making the demand for mint great attraction, moving the trade and allowing opening of new markets.

Despite having high economic importance because of the role it plays in the manufacture of perfumes, peppermint plants also have medicinal, antiseptic, cardiotonic, bleaching hair, collagen, digestive, skin stiffener, stimulant and purgative properties [1, 2]. The availability of raw materials is not sufficient to meet local demand so the products from the peppermint are made by artisans to increase family income. *M. piperita* plays an important role in the economy of the State of Para, precisely in the perfume and cosmetic industries [3], which had increases of about 66.5% due to a demand for aromatic products, and a strong industrial demand of about 2,065 tons.

Despite the intense search for *M. piperita*, there is little information about the nutritional limitations that the plant may show. Blank *et al.* [4] report that there is little information in the literature, with no specific work related to the best nutrient solution that provides the best plant growth. As it is not known which are the most nutrients that could limit *M. piperita* production, it has been used hydroponics to obtain a better response in a short period of time [5, 6]. Hydroponics is a technique where nutrients are supplied by a nutrient solution, which has been widespread in the forage production.

Therefore, one of the major limitations of market expansion is a nutritional issue, and poor investment in research to the needs of the species for nutrients that limit its growth. This culture is very important by its ability of producing essential oil, a major constituent of menthol, used as flavouring, additives, food and oral hygiene products[7]. The objective of this study was to evaluate the growth, symptomatology and dry matter production of *M. piperita*, grown in nutrient solution.

Material and Methods

Experimental conditions: Experiment was carried out in an experimental area of Instituto de Ciências Agrárias ICA) of Universidade Federal Rural da Amazonia, in Belém State of Pará.

Recipients and plant obtaining: Seedlings of *Mentha piperita* L., from the Department of Plant Science were transplanted to plastic pots containing 3 kg of ground quartz substrate (zero coarse, type 4).

Plant material and acclimation: Peppermint plants were daily watered with a complete solution Hoagland *et al.* [8], in a rate 1:1, for a period of 15 days for adaptation (Table 1).

Table 1. Chemical composition of nutrient solutions (mL^{-1}) used in the experiment, according to Hoagland and Arnon [8], modified.

Stock solution	Conc.	C	-N	-P	-K	-Ca	-Mg	-S	-B	-Cu	-Fe	-Mn	-Zn
$NH_4H_2PO_4$	1 M	2	-	-	2	2	2	2	-	-	-	-	-
KNO_3	1 M	6	-	8	-	6	6	6	-	-	-	-	-
$Ca(NO_3)_2$	1 M	4	-	4	4	-	4	4	-	-	-	-	-
$MgSO_4$	1 M	1	1	1	1	1	-	-	-	-	-	-	-
KH_2PO_4	1 M	-	2	-	-	-	-	-	-	-	-	-	-
KCl	1 M	-	4	-	-	-	-	-	-	-	-	-	-
$CaCl_2$	1 M	-	4	-	-	-	-	-	-	-	-	-	-
NH_4NO_3	1 M	-	-	2	-	-	-	-	-	-	-	-	-
$NaNO_3$	1 M	-	-	-	6	4	-	-	-	-	-	-	-
K_2SO_4	1 M	-	-	-	-	-	1	-	-	-	-	-	-
$MgCl_2$	1 M	-	-	-	-	-	-	1	-	-	-	-	-
Solution a*		1	-	-	-	-	-	-	-	-	-	-	-
Solution a - B		-	-	-	-	-	-	-	1	-	-	-	-
Solution a - Cu		-	-	-	-	-	-	-	-	1	-	-	-
Solution - Fe		-	-	-	-	-	-	-	-	-	1	-	-
Solution - Mn		-	-	-	-	-	-	-	-	-	-	1	-
Solution - Zn		-	-	-	-	-	-	-	-	-	-	-	1
Solution de -Fe EDTA**		1	-	-	-	-	-	-	-	-	-	-	-

*Composition of solution: 2.86 g of H_3BO_3; 1.81 g of $MnCl_2.4H_2O$ and 0.22 g of $ZnSO_4.7H_2O$; 0.88 g $CuSO_4.5H_2O$; 0.02 g of H_2MO_3;
**Composition of solution of Fe-EDTA, 26.1 g of Fe-EDTA; 286 ml of 1N KOH and 24.9 g of $FeSO_4.7H_2O$, per litre of solution.
Note: The omission of micronutrient treatments had similar composition to the full treatment and solution, with the exception of solutions -B (B omitted), the -Cu (Cu omitted); a -Mn (Mn omitted) a -zinc (Zn omitted) and in the treatment- Fe omitted solution of Fe-EDTA.

Experimental design: The experimental design was completely randomized with 12 treatments (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn deficiencies, and control), and 5 replicates. These treatments composed of complete nutrient solution for control and omissions of individual nutrients for nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, boron, copper, iron, manganese and zinc.

Qualitative parameters and harvest: The symptoms of deficiencies in macronutrients and micronutrients with the evolution of symptoms and descriptions were made from the first signs of discoloration until symptoms become well established, when plant samples were harvested.

Determination of growth parameters: Plants were sorted out in old leaves, young leaves, stems and roots and washed in distilled water, and dried in an oven with forced air circulation at 70°C, until constant weight. Immediately after getting dry matter, plant material was ground in a Wiley mill for subsequent chemical analysis. Plant height was defined as the distance between the stem, at the substrate level (5 cm) to the apex. The measurement of diameter at 5 cm from substrate was made with a caliper. Leaf area was determined at the end of the experiment, measurements were made with graduated scale. Six leaves were taken at random from two plant strata (old leaves and young leaves), and total twelve samples per plant. In each leaf, length and the greatest width of the blade were measured.

$$\text{Leaf area (AF)} = C \times L$$

where, C is length and L is the greatest width of the blade.

Nutrient determinations: Macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and micronutrients such as boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were determined using methods described by Malavolta *et al.* [9]. Considering data obtained from leaf dry weight and nutrient contents were calculated results of accumulation of nutrients. Low concentration of nutrient induced deficiency symptoms in peppermint tissues were compared to control treatment (C) which contains appropriate amount of nutrients to promote optimal plant growth.

Data analysis: Data were analyzed by computer software Stat for analysis of variance and F-test, and Tukey test at 5% probability, comparing means between treatments on each variable.

Results and Discussion

Visual symptoms and consequences of macronutrient deficiencies:

Nitrogen: The first symptoms of nitrogen deficiency appeared 12 days after initiation of treatment. Initially, peppermint plants showed intense purple colour in old leaves, turning light purple (Fig. 1). It was also observed that the plants had reduced height (34.95 cm) and foliage with small leaf area (113.13 cm^2), and thin stem (0.30 cm diameter), as compared to control treatment. Root system showed long and fine roots. According to Taiz *et al.* [10], symptoms of nitrogen deficiency first appear because it is the most important element

Figure 1. Visual symptoms linked to restriction of nitrogen (B), phosphorus (C), potassium (D), calcium (E), magnesium (F), sulphur (G), boron (H), copper (I), iron (J), manganese (K) and zinc (L) in plants of *Mentha piperita* compared with the full treatment (A) with no deficiency.

required by the plant. The carbohydrate unutilized in the nitrogen metabolism can be used in the synthesis of anthocyanin, leading to accumulation of this pigment which causes the purple colour of leaves and stems of plants deficient in nitrogen. Yellowish colour is a consequence of loss of chlorophyll, and plants with nitrogen deficiency have smaller cells. Batista *et al.* [11] attributed the symptoms of chlorosis in plants with nitrogen deficiency in older leaves firstly, due to its high mobility. Silva *et al.* [12], studying the omission of nitrogen in peach plants found that nitrogen is the first element to induce symptoms of deficiency.

Phosphorus: The omission of phosphorus in plants of *M. piperita* grown in nutrient solution caused the appearance of deficiency symptoms of phosphorus 23 days after initiation of treatment in older leaves, characterized by intense purple color and rough leaves. The stem was rough and coarse, leading to overturning (Fig. 1). The leaves showed necrosis (burning), progressing to the fall and reduced growth in height (67.30 cm), and small leaf area (116.43 cm^2). Plants with thick stem presented 0.65 cm diameter (Table 2). The root system had normal growth and shape. Symptoms similar to those described in this study were also observed by Marques *et al.* [13] on paricá (*Schizolobium amazonicum*, Herb.) cultivated under omission of phosphorus and by Sobral [14] on coconut. Salvador *et al.* [15] attributed the appearance of purplish spots in between veins due to phosphorus deficiency, caused by the accumulation of photoassimilates in plant tissues, promoting synthesis of anthocyanin.

Potassium: Symptoms of potassium deficiency in plants of *M. piperita* L. grown in nutrient solution with potassium omission appeared 25 days after the initiation of treatment and were characterized by marginal chlorosis on older leaves, stiffening of leaves from the edges to inside; and buds with necrosis and whitening of the stem, leading to necrosis in most affected areas (Fig. 1). Potassium deficiency also affected significantly both the height (79.75 cm) and leaf area (59.36 cm^2), but did not affect the diameter (0.63 cm) of peppermint plants (Table 2). Viégas *et al.* [16] observed that the omission of potassium in *Alpinia purpurata* causes the appearance of small orange spots in the old leaves, which coalesces to form larger patches and necrosis followed by blight. Those symptoms are results of accumulation of putrescine which is highly phytotoxic due to non-deployment of nitrogen compounds of the urea cycle caused by lack of potassium. In Fig. 1, a comparison between peppermint plants grown in pots containing full nutrients and with omission of potassium is made.

Calcium: Peppermint plants grown in nutrient solution with the omission of calcium showed deficiency symptoms after 15 days, which is characterized by reduced growth, mainly in aerial part (Fig. 1). The evolution of calcium deficiency gave rise to widespread necrosis and fall of young leaves, bud blight and stem rot. Omission of calcium caused a significant reduction of aerial part (37.02 cm) and leaf area (116.11 cm^2), but did not affect the diameter (0.51 cm) (Table 2). Also root rot was observed especially in younger ones. Leaf area of the plants was smaller than that found by Fazio *et al.* [17] on peppermint. Those results can be explained because in that study different calcium concentrations were supplied in nutrient solution. Freitas *et al.* [18] attributed the reduction of leaves, hence leaf area, to distortions and necrosis that appear when there is lack of calcium.

Symptoms of calcium deficiency observed in peppermint plants are similar to those described by several authors to different cultures. In young plants of teak (*Tectona grandis*), Barroso *et al.* [19] describe the presence of yellowish colour between veins, curling, necrosis of leaves and root rot. Those symptoms were also described by Santos *et al.* [6] in castor bean seedlings, in which hardening of terminal leaves was due to calcium deficiency.

Magnesium: Symptoms of magnesium deficiency in peppermint plants grown in nutrient solution with the omission of magnesium appeared 27 days after initiation of treatment (Fig. 1), characterized by yellowish colour between veins with deep green colour on the tips and margins of the leaves and yellowing between and within veins. In the absence of magnesium significant reduction in plant height (65.17 cm), the leaf area (141.52 cm^2) and the diameter of the stem (0.52 cm) were observed as compared to the complete treatment (Table 2). Santos *et al.* [6] considered the lodging of plants on the stem and reduction in dry matter production as magnesium deficiency.

Sulphur: Peppermint plants grown in nutrient solution with the omission of sulphur presented at 35 days after initiation of treatment, chlorosis and burning in old leaves, with purple coloration between the veins (Fig. 1). Later spots and curving stem appeared and new leaves became with pale green tones and other chlorotic areas. The omission of sulphur significantly affected the development of the aerial part (72.42 cm) and leaf area (163.41 cm^2), but did not affect the diameter (0.62 cm) (Table 2). Symptoms of sulphur deficiency are similar to nitrogen because both nutrients are essential components of proteins, resulting in slower plant growth and stunt [20].

Table 2. Production of dry matter (g) in different organs of *Mentha piperita* plants in different treatments.

Treatment	Plant organs (g planta^{-1})					
	Young leaf (g)	Old leaf (g)	Stem (g)	Aerial part (g)	Root (g)	Total dry matter (g)
Control (C)	9.64 b	59.77 a	45.29 a	114.71 a	14.29 b	129.00 a
N restriction	5.99 d	8.63 e	15.15 e	29.77 e	10.26 c	40.04 f
P restriction	8.26 c	19.80 d	30.48 b	56.05 c	7.27 e	63.33 d
K restriction	7.65 c	27.03c	31.20 b	65.89 b	8.67 d	74.57 c
Ca restriction	5.57 d	9.47 e	22.34 d	37.39 d	8.80 d	46.20 e
Mg restriction	8.44 c	19.27 d	31.09 b	58.80 c	8.59 d	67.39 d
S restriction	12.40 a	30.62 b	26.79 c	69.82 b	16.22 a	86.05 b
B restriction	24.49 d	8.24 de	22.98 d	55.72 e	9.58 d	65.30 e
Cu restriction	26.16 d	10.46 b	32.82 c	69.45 d	12.75 b	82.20 d
Fe restriction	42.27 b	7.9 e	53.01 a	102.48 b	10.63 c	113.12 b
Mn restriction	28.57 c	9.14 cd	31.47 c	69.18 d	12.50 b	81.68 d
Zn restriction	29.30 c	12.80 a	52.19 a	94.30 c	12.69 b	106.99 c

Means followed by the same letters in columns are not significantly different at 5% probability by Tukey test.

Visual symptoms and consequences of micronutrient deficiencies:

Boron: The symptoms of boron deficiency appeared 40 days after starting treatment. It is characterized by chlorosis with burning at the margins of younger leaves (Fig. 1). Plants showed reduced height (35.80 cm), very small leaves and small leaf area (163.73 cm^2). Boron deficiency caused a significant increase in the diameter of plant stems (0.54 cm) as compared to the control treatment (Table 2). Siebeneichler *et al.* [21] studied the pineapple culture and found that the symptoms of boron deficiency are characteristics of young leaves, which present leathery and wrinkled probably due to failures in the structure of the cell wall. The symptoms are similar to those observed in the peppermint culture, which had small and wrinkled leaves. The same has happened in seedlings of jatropha (*Jatropha curcas*), which presented wrinkle at the edges of young leaves with reddish ribs on the underside of older leaves [22].

Copper: Peppermint plants grown in nutrient solution with the omission of copper deficiency manifested symptoms 42 days after initiation of treatment. Initially, the plants showed slight chlorosis between the veins, progressing to necrosis (Fig. 1). We observed a whitening of the stem and the plants showed reduced size when compared with the full treatment (91.93 cm), whose leaves had reduced leaf area (128.51 cm^2). The plants showed thick stem (0.59 cm diameter) in relation to the control (Table 2), and such symptoms are similar to those described by Neves *et al.* [23] on ombúe plants and Camargo *et al.* [24] on *Bertholletia excelsa* plants.

Iron: Symptoms of iron deficiency were observed 46 days after starting treatment. Young leaves became chlorotic, and later red in colour with reddish spots between veins (Fig. 1). At more advanced stages of symptoms, the old leaves also showed chlorosis. The plant height (78.93 cm) and leaf area (129.24 cm^2) were reduced significantly compared to the complete treatment, but there was no effect of the lack of iron in stem diameter (Table 2). Similar symptoms were observed on ombúe (*Spondias tuberosa*), whose iron deficiency leads to alterations in young leaves, stem purple in colour and decrease in chlorophyll level, turning leaves chlorotic and whitish, and causing slow growth [16, 23].

Manganese: The symptoms of manganese deficiency in peppermint plants appeared 47 days after initiation of treatment by means of chlorosis in between ribs and black spots on young leaves (Fig. 1). It was also observed that the plants had small size (59.98 cm), reduced foliage with small leaf area (153.40 cm^2), plant with very thick stem (0.75 cm diameter), as compared with the complete treatment (Table 2). There was thickening of the stem, bending down and cracks. The manifestation of manganese symptoms in peppermint was very similar to the symptoms observed on ombúes, which occurred thickening of the basal portion of the stem, and leaves become chlorotic [23]. Omissions of manganese and zinc did not affect the diameter of the plants [25].

Zinc: The first symptoms of zinc deficiency occurred 47 days of initiation of treatment showed initially chlorosis between veins and fall of the younger leaves. The leaves were well spaced from each other on the stem (Fig. 1). Plants had small size (72.00 cm), reduced foliage with small leaf area (130.74 cm^2) thick stem (0.63 cm diameter), as compared to the complete treatment (Table 2). These plants have been subject to bend, with fissures. There were bending and cracking of the plant stems. Zinc deficiency caused morphological changes in young leaves, characterized by small and narrow leaves, sometimes distorted with short internodes and chlorotic areas in between veins [23], which were observed in this study with peppermint.

Deficiency effects on plant growth and dry matter production:

The largest reductions in plant growth were observed in plants grown under omission of nitrogen, calcium, boron and manganese as compared with the full treatment (Table 2). The omission of manganese and zinc caused an increase in stem diameter compared to the control treatment diameter was (0.43 cm). Batista *et al.* [11] and Lange *et al.* [26] observed a reduction in growth of soursop and castor bean, with the omission of zinc and manganese. The smaller leaf area were observed in plants under omission of copper, iron and zinc. Leaf area was the most affected with the omission of potassium, nitrogen and calcium with values of 59 cm^2, 113.13 cm^2 and 116.11 cm^2. Similar results were shown by Fasabi [27] with mallow plants, variety BR-01.

The values of total dry matter present in different organs of *M. piperita* according to the treatments in Table 2 show that the total dry matter regarding the omission of sulphur in young leaves (12.40 g kg^{-1} of plant) and roots (16.22 g kg^{-1} of plant) was superior to control treatment, except nitrogen and calcium that limited more the production of dry matter in the plant, consistent with the results of Silva Júnior *et al.* [25] and Freitas *et al.* [18].

Conclusions

Nitrogen and calcium are the first to express nutrient deficiency symptoms, followed by phosphorus, potassium, magnesium, sulphur, boron, copper, iron and manganese = zinc. For omission of nitrogen, phosphorus, potassium, magnesium, sulphur, boron, copper, iron, manganese and zinc compromise the growth of *M. piperita* inducing morphological changes with characteristic visual symptoms of deficiencies of these nutrients. The most limiting nutrient for growth of *M. piperita* based on total dry matter and leaf area is nitrogen, followed by calcium, as well as the nutrient that most affects the growth of *M. piperita* is boron. In relation to stem, aerial part and total dry matter the omissions of nitrogen, calcium, boron and copper were most limiting, and in the root the omission of phosphorus and boron was the most outstanding.

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