Growth, chlorophyll content, and visual symptoms of noni (*Morinda citrifolia* L.) seedlings affected by macronutrient deficiency

Crecimiento, contenido de clorofila y síntomas visuales en plántulas de noni (*Morinda citrifolia* L.) afectadas por deficiencia de macronutrientes

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ABSTRACT

Morinda citrifolia L. is a plant utilized for its food and medicinal benefits. However, a lack of information on its nutrient requirement limits the yield potential of this crop for commercial cultivation. Therefore, the response of noni seedlings to the application of complete nutrient solution with or without omission of one macronutrient (to determine the most limiting one) was studied. The treatments consisted of 7 nutrient solutions (Complete Nutrient Solution (CNS) and CNS minus (-) each of N, P, K, Mg, Ca, and S) and four application volumes (0 ml (control), 100 ml, 200 ml, and 300 ml NS) arranged in a randomized complete block design with four replicates in a greenhouse. Data on stem height, stem diameter, number of leaves, leaf area, and visual nutrient deficiency symptoms were assessed at four weeks intervals, starting from the 4^{th} week after transplanting (WAT) in sand culture. Total dry matter yields and leaf chlorophyll content were determined at 20 WAT. The seedlings treated with CNS presented the highest growth in terms of all the variables, with no deficiency symptoms, whereas seedlings treated with CNS-N had the least growth, chlorotic leaves, and a stunted appearance throughout the period of the study. Overall, the order of growth limitation in seedlings was as follows: -N>-Ca>-Mg>-P>-K>-S>CNS. The seedlings treated with 100 ml NS had the best performance as compared to the control and other treatments. These results indicated that N followed by Ca and Mg are the most limiting macronutrients for noni seedling development and are required in relatively small quantities.

Key words: mineral nutrition, deficiency symptoms, fruit crop, nitrogen.

RESUMEN

Morinda citrifolia L. es una planta utilizada por sus beneficios alimentarios y medicinales. Sin embargo, la falta de información sobre sus requerimientos de nutrientes limita el potencial de rendimiento de este cultivo para el cultivo comercial. Por lo tanto, se investigó la respuesta de las plántulas de noni a la aplicación de una solución nutritiva completa, con o sin la omisión de un macronutriente (para determinar el más limitante). Los tratamientos consistieron en 7 soluciones nutritivas (Solución Nutritiva Completa (CNS) y CNS menos (-) cada uno de N, P, K, Mg, Ca y S) y cuatro volúmenes de aplicación (0 (control), 100, 200 y 300 ml NS) dispuestos en un diseño de bloques completos al azar con cuatro repeticiones en invernadero. Los datos sobre altura y diámetro del tallo, número de hojas, área foliar y síntomas visuales de deficiencia de nutrientes se evaluaron en intervalos de cuatro semanas, a partir de la cuarta semana después del trasplante (SDT) en cultivo de arena. Los rendimientos totales de materia seca y el contenido de clorofila de las hojas se determinaron a las 20 SDT. Las plántulas tratadas con CNS tuvieron el mayor crecimiento en términos de todas las variables consideradas sin síntomas de deficiencia, mientras que las plántulas tratadas con CNS-N tuvieron el menor crecimiento, mostrando hojas cloróticas y apariencia atrofiada durante todo el período del estudio. En general, el orden de limitación del crecimiento en las plántulas fue: -N>-Ca>-Mg>-P>-K>-S>CNS. El rendimiento de las plántulas bajo la aplicación de 100 ml de NS fue el mejor en comparación con el control y otros tratamientos. Estos resultados indicaron que el N, seguido del Ca y Mg, son los macronutrientes más limitantes para el desarrollo de las plántulas de noni y se requieren en cantidades relativamente pequeñas.

Palabras clave: nutrición mineral, síntomas de deficiencia, frutal, nitrógeno.

Received for publication: August 1, 2024. Accepted for publication: October 7, 2024. Doi: 10.15446/agron.colomb.v42n3.116051

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Introduction

Noni (*Morinda citrifolia* L.) is an evergreen fruit-producing shrub, valuable for many health benefits and derived products (Sadino *et al*., 2024). Its fruit juice is widely consumed as nutritional tonic, while noni leaves, stem, bark, roots and flowers are used for medicinal purposes owing to their therapeutic properties (Ali *et al*., 2016). The species belongs to the Rubiaceae family and is characterized by white tubular flowers, large, bright green glossy leaves and yellowish fruits (Monroy *et al*., 2021). The species tolerates different types of soil and can grow under variable climatic conditions (Natarajan *et al*., 2023; Souto *et al*., 2016; Yashaswini *et al*., 2014). However, the fruit production, fruit quality and the nutritional composition vary among the varieties or genotypes and are directly related to the environment as well as to the cultivation system (Arya *et al*., 2022; Basar & Westendorf, 2012; Deng *et al*., 2010).

In fruit species, the lack or deficiency of essential nutrients is among the factors which can limit growth during vegetative stage and affect subsequent fruit production (Iqbal *et al*., 2023). Soil supplies most of the essential elements required by plants for optimum growth (Njinga *et al*., 2013; Toor *et al*., 2021). The availability of these elements is affected by geographic location, weather conditions, terrain, and other factors (Singh & Schulze, 2015; Wang *et al*., 2002). Tropical regions are commonly characterized by abundant and frequent rainfall, intense vertical sunlight exposure, and consistent warm temperatures, which often leads to leaching of mineral nutrients from soil and high rates of organic matter decomposition (Payne & Edis, 2012; Taylor *et al*., 2017). Under such conditions, availability and supply of some nutrient elements becomes unreliable, thus, limiting the growth of plants, especially fruit crops such as noni, under intensive cultivation. Noni has been observed to display abnormal foliar symptoms, which could be due to depletion of mineral nutrients under continuous fruit production and prevailing conditions of the tropical soils (Honey *et al*., 2012; Nelson & Elevitch, 2006). Thus, improving production and crop yield requires an appropriate fertilizer application program.

Up until now, fertilizer applications in the form of organic, mineral, and biofertilizers have been reported for noni (Caione *et al*., 2018; Sahoo *et al*., 2017). However, due to lack of information on the nutritional requirements of the species, some applications were made based on the requirements of related species (*e.g.,* coffee), while others placed emphasized on K as one of the most abundant elements in noni fruit (Melo *et al*., 2021; Souto *et al*., 2018). The basic application of fertilizer to noni plants without considering the most limiting element disregards the law of the minimum, which relates the yield level to the nutrient in lowest (minimum) supply (Brown *et al*., 2022). Moreover, the rate at which crops utilize nutrients differ between inter and intra species (Adhikari *et al*., 2023; Morgan & Connolly, 2013). Hence, this creates an ample scope for the determination of the specific limiting nutrient for noni growth. Availability of such knowledge in combination with information on soil nutrient status would provide a basis for fertilizer recommendation for this plant. Therefore, the response of noni seedlings to the application of complete nutrient solution with or without omission of one macronutrient (to determine the most limiting one) was studied in the present research.

Materials and methods

Study site

This study was conducted in a glass greenhouse of the Biotechnology Department, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo - State, Nigeria, located at longitudes 07º23'18"N to 07º23'43"N and latitudes 03º51'20"E to 03º23'43"E. The greenhouse was situated at 215 m a.s.l. The weather reports of the location were obtained from FRIN Meteorological station and indicated average maximum and minimum temperatures of 32.4°C and 23.6°C, respectively. The mean relative air humidity was 79.2%, while mean monthly rainfall was 114 mm during the period of the study from March to August, 2023.

Treatments and experimental design

This experiment consisted of 7×4 factorial treatment combinations. The first factor was the type of nutrient solution (NS): complete nutrient solution (CNS) with macro and micronutrients, CNS minus (–) each of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and sulphur (S). The second factor was four volumes of nutrient solution: 0 (control), 100, 200, and 300 ml. These volumes per plant were applied twice a week. The control did not receive a nutrient solution, and 200 ml of distilled water per plant was applied in the control treatment. Application volumes varied in order to expose the plants to adequate nutrient concentrations, as there were no reports establishing a particular volume of Hoagland solution for the optimum growth of the noni seedlings. The resulting 28 treatments were laid out in a randomized complete block design with four replicates, where each replicate corresponded to one plant per pot.

Growth medium preparation

Sand culture and "minus one element technique" (MOET) were used for this experiment (Burns, 1992). The river sand used was collected from the Asanmagbe riverbank in FRIN. The sand was sieved to remove particles larger than 4.5 mm, washed several times under flowing tap water until the water became clear, and then washed with distilled water. The sand was then leached with 2 N HCl solution containing 1% oxalic acid for 18 h and later washed with deionised water (Taxiarchou *et al.*, 1997). It was sterilized at 100°C for 1 h in a hot air oven and was filled into polythene pots of 2 kg size, perforated at the base.

Preparation of the nutrient solutions

Stock solutions of the essential macronutrients were prepared, and the treatment solutions were made by adding the correct proportions of the required stock solutions according to Hoagland and Arnon (1938), as shown in Table 1.

Initial growth and transplanting of seedlings

Mature noni (*Morinda citrifolia* var. citrifolia) fruits were collected from the FRIN arboretum. The seeds were extracted and sundried. Seeds of similar size were sorted, clipped at the tip and propagated in leached, sterilized river-sand substrate at 100 seeds per basket. Seedlings began to emerge three weeks after sowing. Seedlings of uniform height at the four-leaf stage were carefully removed, their roots rinsed and transplanted into the growth media (sand) at one seedling per pot. The seedlings were watered with distilled water for the first week, followed by the application of 50% complete Hoagland solution during the second week to stabilize the plants. Subsequently, nutrient solutions according to each treatment were applied by the slop method, twice a week (McCall & Nagakawa, 1970). A plastic plate was placed under each pot to prevent outside root extension, while leachate was not reused.

Data collection

The biometric response of the plants in terms of stem height (cm), stem diameter (mm), number of leaves, and leaf area (cm^2) was assessed at four-week intervals, starting from the 4th week after transplanting (WAT). Total dry matter (shoot + roots) (g/plant) and leaf chlorophyll content were determined at 20 WAT. The seedlings were carefully uprooted, the sand rinsed off the roots and then separated into leaves, stems and roots. The plant parts were then put in separate envelopes, labelled according to treatments and oven dried to constant weight at 70°C in an electric oven. The oven-dried weights of each of the plant parts were added together to obtain the total dry biomass. Nutrient deficiency symptoms in the plants were visually assessed along with the collection of biometric data. Stem height was determined with a meter rule. Stem diameter at the base was evaluated using a digital vernier calliper. Leaf area was measured with a portable leaf area meter (YMJ-B®, Hinotek), while the number of leaves was assessed by counting. Relative contents of chlorophyll (SPAD units) were measured in a third or fourth pair leaf from the top with a hand-held chlorophyll meter (TYS-B®, Hinotek).

Statistical analysis

The data collected were subjected to analysis of variance using GenStat (version 4), while the significantly different means were separated using the Duncan Multiple Range Test at 5% probability.

TABLE 1. Composition of the "minus one element culture" solution.

Stock solutions	Molarity (M)	Complete solution (ml L^{-1})	Stocks to be added to omit an element (ml L^{-1})					
			-N	-P	-K	-Ca	-Mg	-S
KNO_3	1	5	-	6	-	5	6	6
KH_2PO_4	1	1	-	-	-	1	1	1
$Ca(NO_3)_2 \cdot 4H_2O$	1	5	-	4	5	-	4	4
$MgSO_4 \cdot 7H_2O$	1	2	2	2	2	2	-	-
$CaSO_4 \cdot 2H_2O$	0.01	-	200	-	-	-	-	-
K_2SO_4	0.5	-	5	-	-	-	3	-
$Ca(H_2PO_4)_2 \cdot H_2O$	0.05	-	10	-	10	-	-	-
$Mg(NO_3)_2 \cdot 6H_2O$	1	-	-	-	-	-	-	2
Iron solution		1	+	+	+	+	+	+
Trace element solution		1	+	+	+	+	+	+

+ indicates presence and - indicates absence of a particular mineral element.

Ferric-sodium salt of EDTA (18.4 g L^{-1}) was used to replace Fe tartrate in the Hoagland solution. Trace element solution consisted of H_3BO_3 (2.86 g), $MnCl_2 \cdot 4H_2O$ (1.81 g), $ZnSO_4 \cdot 7H_2O$ (0.22 g), $CuSO_4 \cdot 5H_2O$ (0.08 g), and $H_2MoO_4 \cdot H_2O$ (0.02 g) in 1 L of distilled water.

Results

Plant height

The growth of noni seedlings, as affected by different nutrient solutions, indicated significant differences ($P \leq 0.05$) between the treatment means for plant height at 8 to 20 weeks after transplanting (WAT) (Fig. 1A). Moreover, results at 12, 16, and 20 WAT followed a similar pattern, except for the plants treated with CNS-Ca. Seedlings treated with complete nutrient solution CNS had the highest mean heights of 20.4, 34.7, and 43.0 cm at 12, 16, and 20 WAT, respectively, which were significantly taller than the seedlings in each of the other treatments at the respective growth periods. These were followed by seedlings treated with CNS-S, which were taller than seedlings of other treatments, except the CNS treatment, at the same periods. The plant height in the CNS-Ca treatment decreased gradually towards 20 WAT. The smallest heights of 7.4, 8.7, and 9.7 cm at 12, 16, and 20 WAT, respectively, were observed in seedlings treated with CNS-N (Fig. 1A).

Stem diameter

The analysis of variance revealed significant differences ($P \leq 0.05$) between the nutrient solutions for stem diameter at 8, 12, 16, and 20 WAT. Seedlings treated with CNS had significantly higher stem diameter compared to those from other treatments throughout the period of study (Fig. 1B). At 8 WAT, the stem diameter of seedlings treated with CNS-Mg, CNS-S, and CNS-Ca were comparable, with each significantly higher than the stem diameter of seedlings treated with CNS-K and CNS-N. The smallest stem diameter was observed in seedlings treated with CNS-P at 8 WAT. At 12 and 16 WAT, the seedlings treated with CNS-S had thicker stems than seedlings treated with CNS-Ca. Similarly, the stem diameter of seedlings treated with CNS-K and CNS-P were similar and significantly higher than that of seedlings treated with CNS-N, with the smallest values at 12 and 16 WAT. At 20 WAT, the stem diameter of seedlings subjected to CNS-S was significantly higher than in the other treatments, while the seedlings treated with CNS-N had the smallest stem diameter (Fig. 1B).

Number of leaves

The number of leaves of noni seedlings treated with various NS significantly differed ($P \leq 0.05$) among the treatments from 8 to 20 WAT (Fig. 1C). The seedlings treated with CNS-Ca produced the highest number of leaves at 8 WAT, while those supplied with CNS-N had the least number of leaves. At 12 WAT, the seedlings treated with CNS had the highest number of leaves, whereas seedlings treated with CNS-Mg had the least number of leaves. Moreover, the number of leaves produced by seedlings treated with CNS-Mg decreased gradually from 8 to 20 WAT, while the number of leaves in the CNS-Ca treatment decreased from 12 WAT onward, reaching the lowest values at 16 and 20 WAT. At 16 and 20 WAT, the seedlings treated with CNS produced the highest number of leaves, closely related to those treated with CNS-K, and significantly higher than the number of leaves for other treatments at 20 WAT (Fig. 1C).

Leaf area

Significant variations ($P \leq 0.05$) in leaf area were found between the treatment means at successive weeks of growth, except at 4 WAT. At 8 WAT seedlings treated with CNS-Ca had the highest leaf area (135.9 cm^2), which was not significantly different from values observed for seedlings treated with CNS (133.1 cm^2), CNS-Mg (123 cm^2) or CNS-S (117.7 cm^2) (Fig. 1D). The smallest leaf area (52.9 cm^2) during this period was observed in seedlings treated with CNS-P, which was closely related to the value obtained for seedlings treated with CNS-N. At 12 WAT, the seedlings treated with CNS had significantly higher leaf area compared to other treatments. Seedlings treated with CNS-N had the smallest leaf area at 12 WAT. Results at 16 and 20 WAT followed a similar trend. Seedlings treated with CNS had the highest leaf area at 16 and 20 WAT (1097.7 and 1363.5 cm^2, respectively), significantly exceeding the leaf area of other treatments at both periods. The seedlings treated with CNS-N had the smallest leaf area at both periods. The leaf area of the seedlings treated with CNS-Ca declined gradually from 12 WAT to 20 WAT (Fig. 1D).

Total dry matter accumulation

The analysis of variance indicated that the effects of NS, application rates, and their interactions were highly significant ($P \leq 0.01$) on the total dry matter yields of noni seedlings at 20 WAT. Seedlings treated with CNS had the highest total dry matter (15.45 g/plant), which was significantly higher than values for each of the other treatments (Fig. 2A). This was followed by seedlings treated with CNS-S with 8.08 g/plant. The dry matter of seedlings treated with CNS-K (5.42 g/plant) was similar to that of seedlings treated with CNS-P (4.40 g/plant), which was also not different from the dry matter of seedlings treated with CNS-Mg (3.36 g/plant). Moreover, the dry weight of seedlings treated with CNS-Mg was closely related to those of the CNS-Ca treatment (2.65 g/plant). The least biomass (1.52 g/plant) was observed in seedlings treated with CNS-N at the same period (Fig. 2A). Between the NS volumes, the seedlings under the least application volume (100 ml NS) accumulated the highest dry matter (8.91 g/plant), which was significantly higher than the seedling

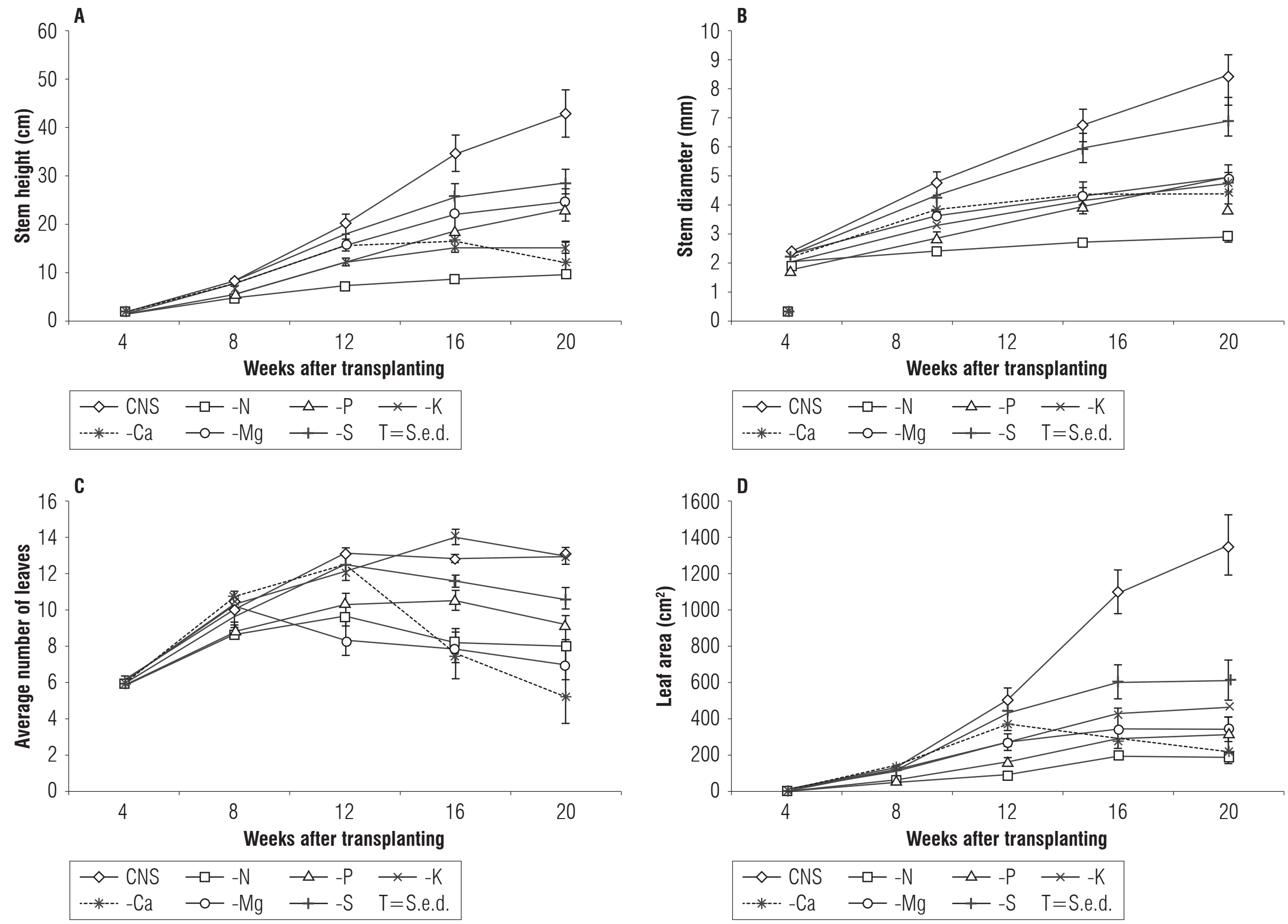

FIGURE 1. Effects of the nutrient solutions on (A) plant height, (B) stem diameter, (C) number of leaves, and (D) leaf area of noni seedlings during 20 weeks after transplanting into sand culture. Error bars correspond to standard error (n = 16). CNS - complete nutrient solution.

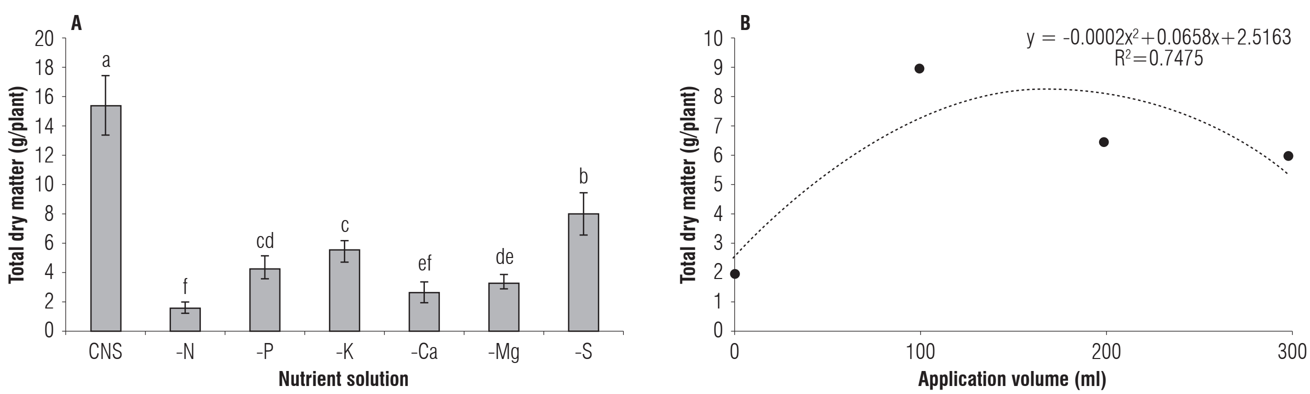

FIGURE 2. Effects of (A) nutrient solutions and (B) application volumes on total dry matter of noni seedlings at 20 weeks after transplanting into sand culture. Means with different letters indicate significant differences according to the Duncan test ($P \leq 0.05$). Error bars correspond to standard error. CNS - complete nutrient solution.

weights obtained for each of the other volumes (200 or 300 ml of NS) and control which had the least seedling weight at 20 WAT (Fig. 2B).

Furthermore, results of the effects of nutrient solution volumes on plant height, stem diameter and leaf area showed significant differences ($P \leq 0.05$) for means of the variables at successive weeks of growth (Fig. 3). Seedlings treated with 100 ml NS had the highest plant height, stem diameter and leaf area, while the control seedlings had the least values for these variables at 8 to 20 WAT. Conversely, the control seedlings had the highest number of leaves, while seedlings

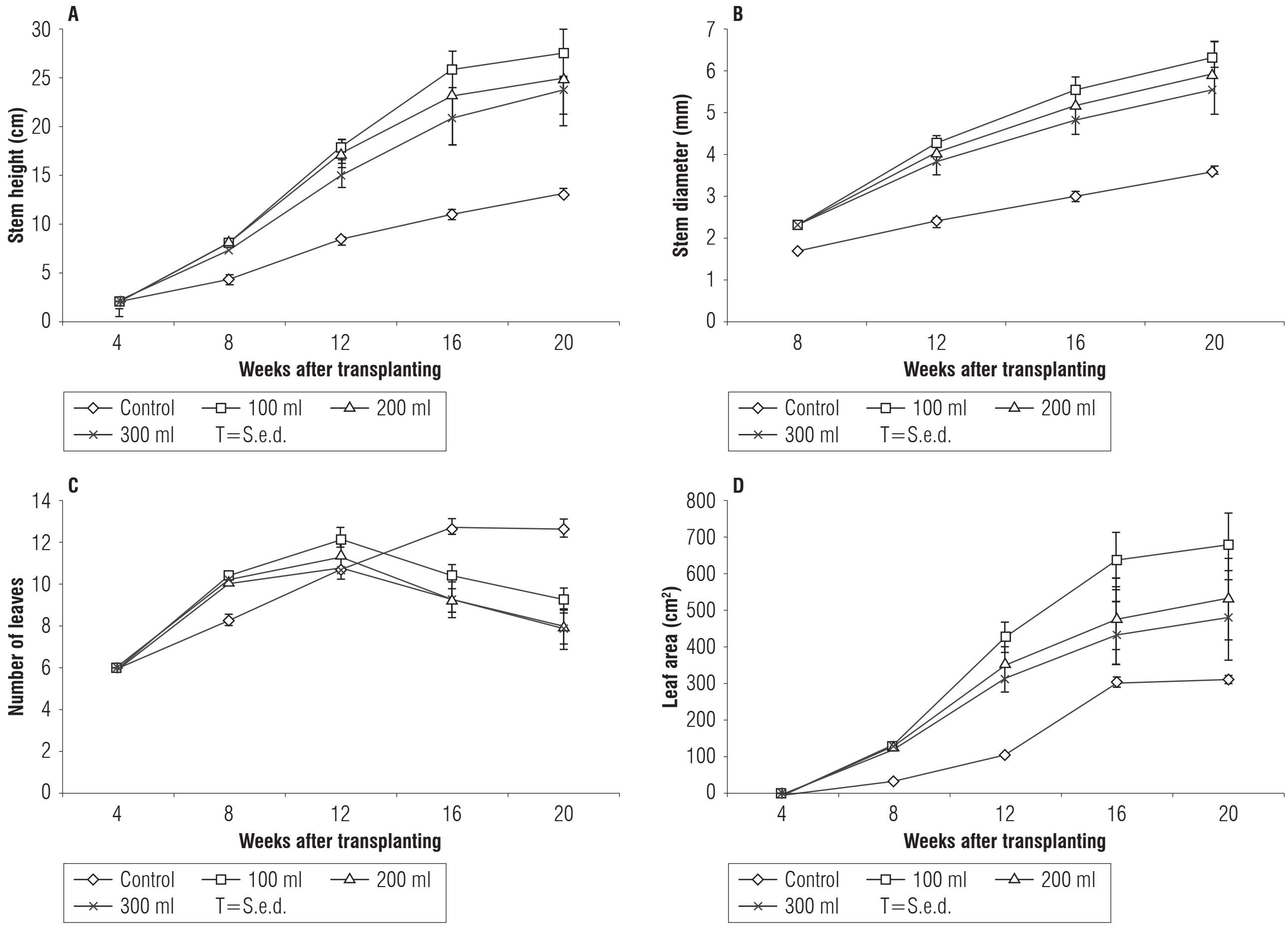

FIGURE 3. Effects of nutrient solution application volumes on (A) plant height, (B) stem diameter, (C) number of leaves, and (D) leaf area of noni seedlings during 20 weeks after transplanting into sand culture. Error bars correspond to standard error (n = 28). Control - distilled water.

treated with 100 ml, 200 ml, or 300 ml of NS decreased in number of leaves from 12 to 20 WAT (Fig. 3C). The results of the interactive effects of NS and application volumes on the seedlings also revealed significant differences for means of all variables at successive weeks of growth.

Leaf deficiency symptoms

Observations on leaf deficiency symptoms of noni seedlings indicated that plant growth was affected by the different NS treatments (Fig. 4). The growth of the seedlings was uniform with no visible deficiency symptoms at 4 WAT. However, at 8 WAT, seedlings treated with nutrient-deficient solutions began to show deficiency symptoms, which persisted and became severe at 16 and 20 WAT (Fig. 4). Seedlings treated with CNS showed no deficiency symptoms throughout the periods of observation (Fig. 4A). In contrast, stunted growth and chlorosis, which started in the older leaves and progressed to the younger leaves, were observed in seedlings treated with CNS-N (Fig. 4B). Similarly, small leaves and retarded growth were identified in seedlings treated with CNS-P (Fig. 4C), whereas brown patches in the third pair of leaves and crinkled stems were observed in seedlings treated with CNS-K (Fig. 4D). Seedlings treated with CNS-Ca produced interveinal chlorosis in young fully expanded leaves, burned leaf margins, and shoot tips die-back (Fig. 4E). Similarly, necrosis and premature leaf fall were noticed in seedlings treated with CNS-Mg (Fig. 4F), while seedlings treated with CNS-S had interveinal chlorosis on young and recently matured leaves (Fig. 4G) compared with seedlings treated with CNS at the same growth period.

Chlorophyll content

The chlorophyll (CHL) content in the leaves of noni seedlings varied significantly with respect to different nutrient solutions applied (Fig. 5). The seedlings treated with CNS had the highest CHL content (71.7 SPAD value), which was significantly higher than values for each of the other treatment solutions at 20 WAT (Fig. 5A). The lowest CHL content was recorded for seedlings treated with CNS-Ca at

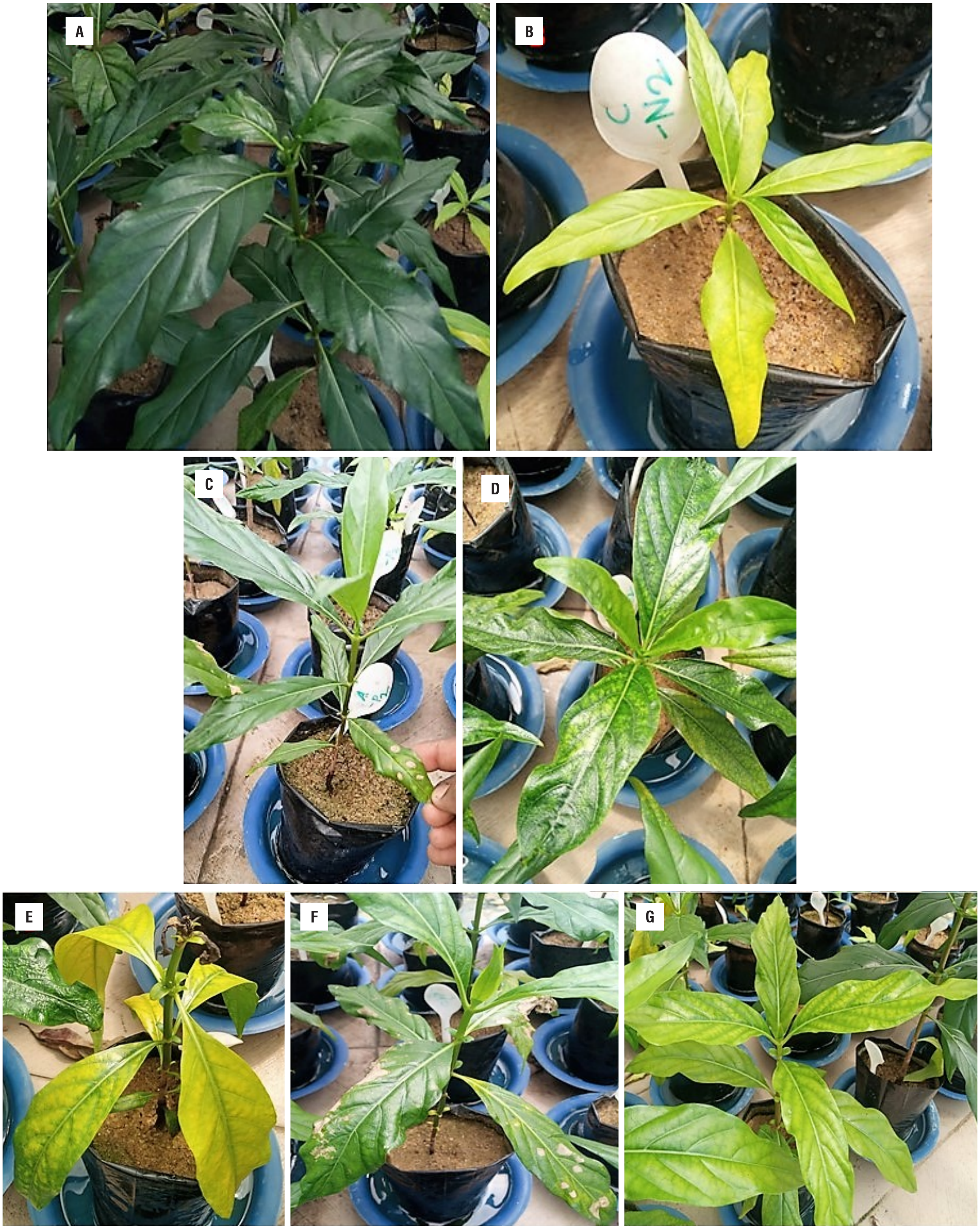

FIGURE 4. Macronutrient deficiency symptoms in noni seedlings at 16 weeks after transplanting into sand culture as affected by nutrient solutions (200 ml applied twice a week). The seedlings were treated with: A) Complete Nutrient Solution (CNS); B) CNS – N; C) CNS – P; D) CNS – K; E) CNS – Ca; F) CNS – Mg; and G) CNS – S.

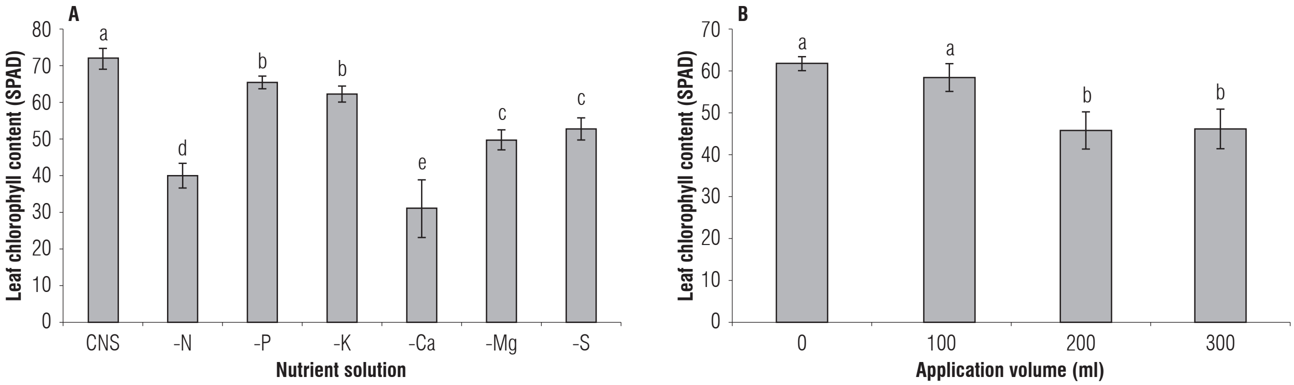

FIGURE 5. Effects of (A) nutrient solutions and (B) application levels on leaf chlorophyll content of noni seedlings at 20 weeks after transplanting into sand culture. Means with different letters indicate significant differences according to the Duncan test ($P \leq 0.05$). Error bars correspond to standard error. CNS - complete nutrient solution.

20 WAT. The CHL content in ascending order was CNS-Ca < CNS-N < CNS-Mg < CNS-S < CNS-K < CNS-P < CNS (Fig. 5). The CHL content was also affected by the levels of NS applied (Fig. 5B). The seedlings treated with only distilled water had CHL contents similar to those treated with 100 ml NS. The CHL values for these treatments were significantly higher than those recorded for seedlings treated with 200 ml or 300 ml NS at 20 WAT.

Discussion

The highest growth was observed in seedlings watered with complete nutrient solution in terms of plant height, stem diameter, number of leaves, leaf area, and total dry matter, which could be attributed to the availability of all the essential nutrients in the CNS used (Hoagland & Arnon, 1938). These results indicated that the concentration of the essential nutrients in the CNS, even at the least application volume (100 ml NS), was sufficient for normal growth in noni seedlings while providing a basis for comparison with other treatment solutions applied in the present study. Moreover, the better growth observed at the lowest application volume could be attributed to the higher efficiency of the photosynthetic apparatus used by the plants (Lambers *et al*., 2008). This was evident in the highest values of leaf area, total dry matter, and chlorophyll content observed for the least NS volume compared with control and other volumes (200 ml and 300 ml of NS). This indicated that noni seedlings might not require a high nutrient dose but the balanced nutrition for optimum growth.

Essential elements are needed for optimum growth serving as integral parts of plant metabolic pathways. Their functions cannot be substituted by another element, and without them no plant can complete its life cycle (Toor *et al*., 2021). These assertions were obvious in the results obtained for seedlings treated with CNS deficient in a macronutrient (N, P, K, Ca, Mg, and S). Generally, the pattern of growth of seedlings under each of the deficient nutrient solutions followed Liebig's "law of the minimum" which states that the nutrient in shortest supply determines the growth or yield level (Kihara *et al*., 2022). Consequently, the absence of an essential macronutrient in each of the solutions might have deprived the plant of necessary metabolic activities, resulting in retarded and suppressed growth observed in the seedlings compared with those under CNS application. Hitherto, there are reports on fertilizer application to noni plants (Caione *et al*., 2017), but studies on nutrient omission study are lacking. Hence, these results could be related to those of Costa *et al*. (2024), where complete Hoagland and Arnon nutrient solution was applied to coffee plants (a species related to noni) to obtain leaves with contrasting elemental compositions.

Nitrogen is involved in the formation of amino acids, proteins, chlorophyll, and vitamins in plants (Fathi, 2022). Lack of these functions might have caused the short, thin plants and overall chlorosis observed in noni seedlings treated with CNS-N. Moreover, the least growth observed in the same seedlings in terms of plant height, stem diameter, leaf area, and total dry matter at successive weeks after transplanting indicated that nitrogen is the most limiting macronutrient for the optimum growth of noni at seedling stage. Starting from 8 WAT, the seedlings in CNS-N were underdeveloped, and this phenomenon persisted until the end of the study. Similarly, the poor growth observed in the seedlings subjected to the CNS-Ca treatment at 20 WAT underscores the importance of Ca in formation of the cell wall middle lamella and cell membranes (Thor, 2019). Initially, rapid growth was observed in the CNS-Ca seedlings up to 12 WAT. However, the malformed new leaves, low chlorophyll content, cupped leaves, burned tips, and stem die-back at later stages (16 to 20 WAT) could be attributed to the absence of Ca in the nutrient solution. Calcium influences N uptake, cell growth, and cell division as well as water movement in plants (Ramírez-Builes *et al.*, 2020). Deficiency of these functions might have caused the reduced growth observed in the seedlings, even at higher concentrations of other nutrients, considering Ca as the second most limiting nutrient for noni growth after N. These results could be related to those of Flores *et al.* (2016), where omission of N and Ca among other deficient nutrients (P, K, Mg, and S) mostly affected the growth of coffee plants in terms of lower dry matter accumulation; therefore, nitrogen was selected as the most required mineral element in coffee at this stage of growth. In another study, N followed by Ca were also reported as the most demanded macronutrients in *Coffee canephora* (Ramírez-Builes *et al.*, 2020).

Next to Ca, Mg is the limiting macronutrient for noni growth, as revealed by the results of this study. The necrosis observed in the seedlings treated with CNS-Mg was specific to the treatment throughout the periods of study and was indicative of Mg deficiency (Fig. 4F). Magnesium is a central cation in chlorophyll molecules and acts as an enzyme activator. It is required for stabilization of nucleic acids and ATP formation (Kathpalia & Bhatla, 2018). The deficiency of Mg in mature leaves of seedlings treated with Mg-deficient solution might have led to reduced photosynthetic activities and reduced influx of mineral nutrients through xylem (Tanoi & Kobayashi, 2015). This, in turn, might have caused necrosis and defoliation, culminating in the reduced total dry matter in the CNS-Mg seedlings compared with those treated with CNS at 20 WAT. On the other hand, the deficiency symptoms observed in the present study with nutrient solutions deficient in each of P, K and S proved that each of these macronutrients is essential for optimum growth of noni seedlings. However, the requirement for these nutrients by noni is not as important as that of N, Ca and Mg (Subramanian *et al.*, 2023). This was evident in better growth observed for the seedlings under application of the solutions deficient in each of P, K, and S compared with seedlings to which solutions deficient in each of N, Ca, and Mg was applied.

Conclusions

Noni seedlings under various deficient macronutrient solutions exhibited different deficiency symptoms and growth limitations. Nitrogen, followed by Ca and Mg, are the most limiting macronutrients required for optimum growth of noni at vegetative stage. These nutrients are also required in relatively small amounts, considering the best performance of noni seedlings under the least level of nutrient solution applied. Consequently, the present study has provided an insight into the most required macronutrients for noni, and the results should serve as a guide for fertilizer recommendation for the species.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

JOA and EAA formulated the overarching research goals and aims. All authors designed the methodology. JOA conducted the research, data/evidence collection and statistical analysis. JOA wrote the initial draft. AOO contributed to the results presentation. AEA oversaw and led the research activity planning and execution. All authors reviewed the final version of the manuscript.

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